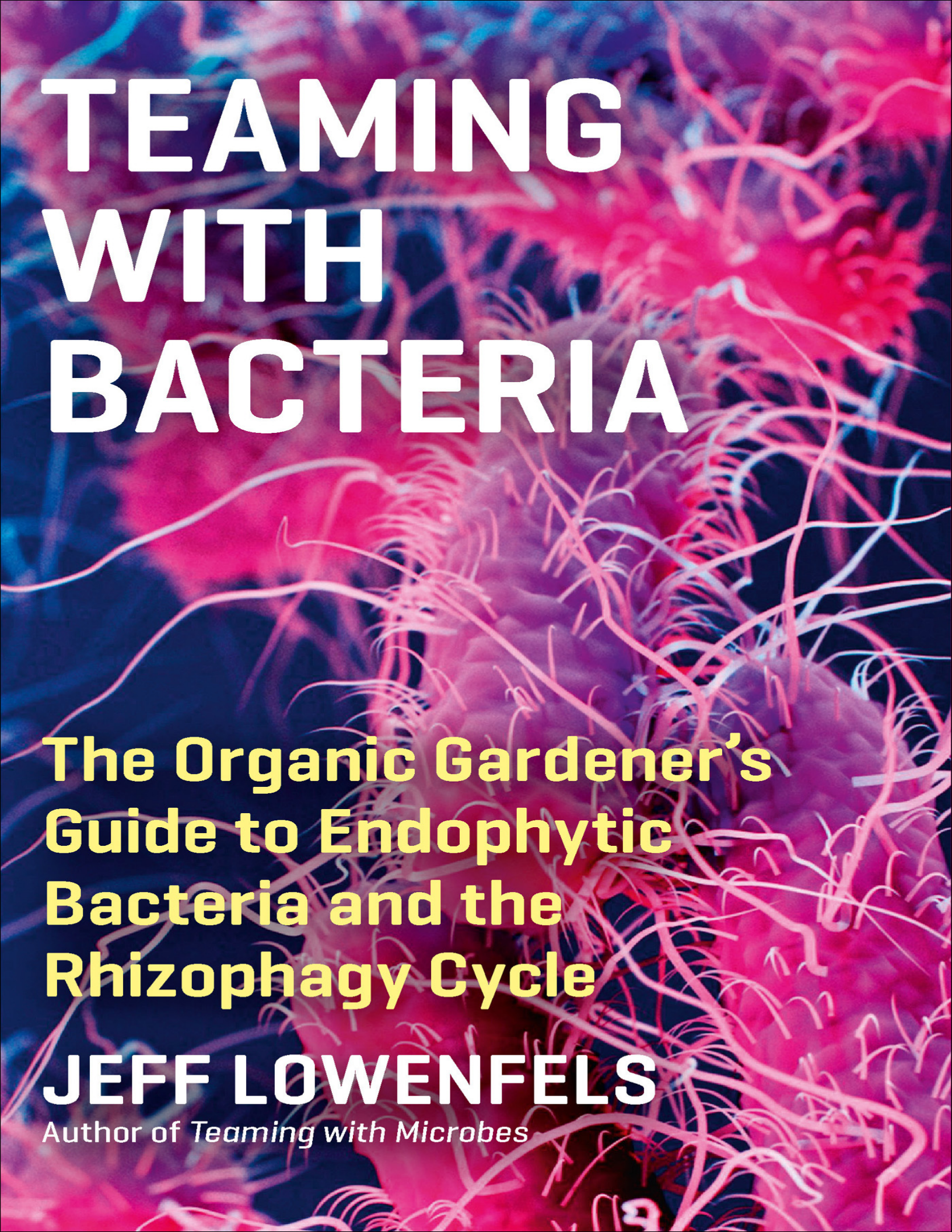
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The Organic Gardener's Guide to Endophytic Bacteria and the Rhizophagy Cycle
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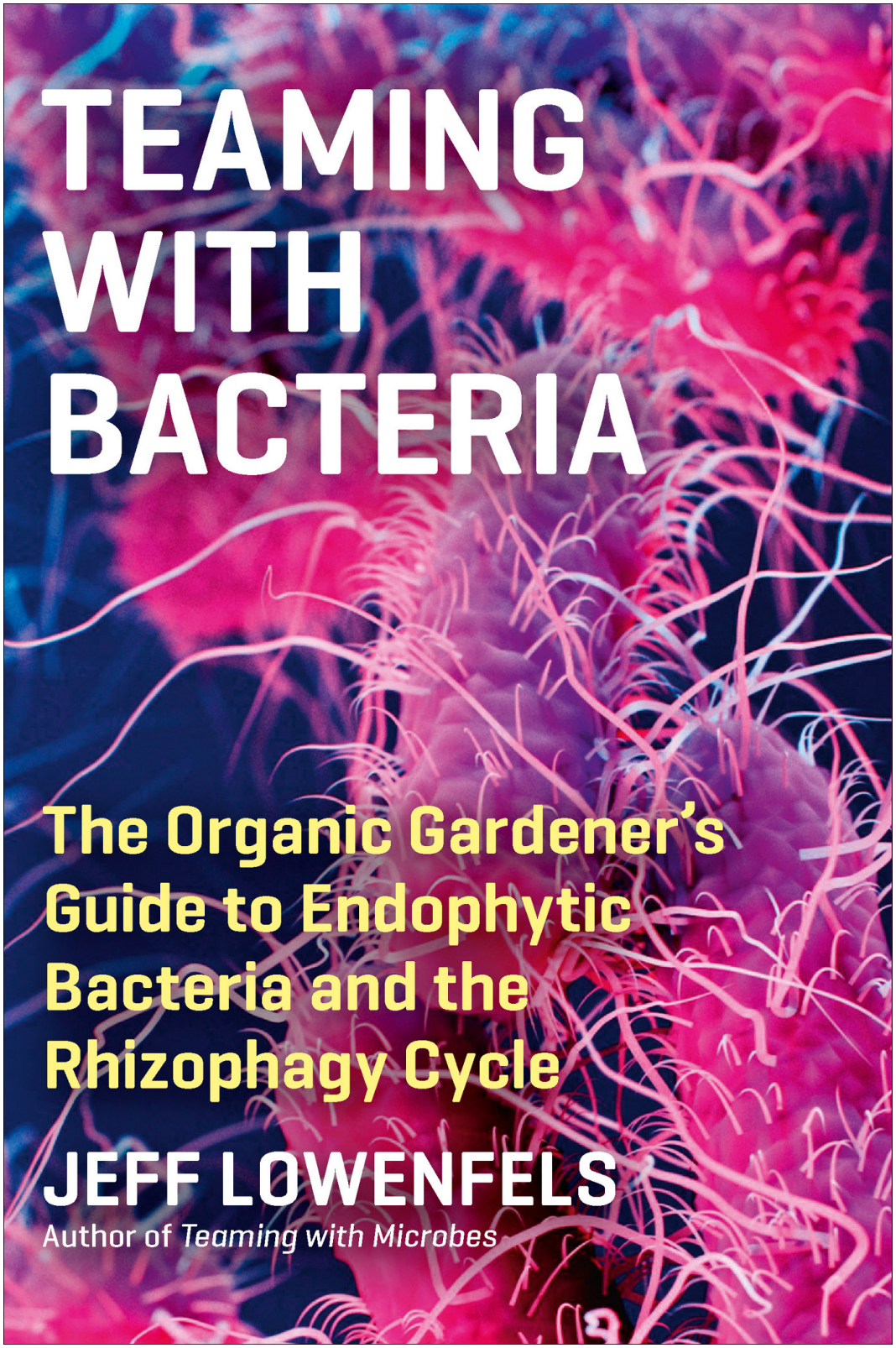
Author of Teaming with Microbes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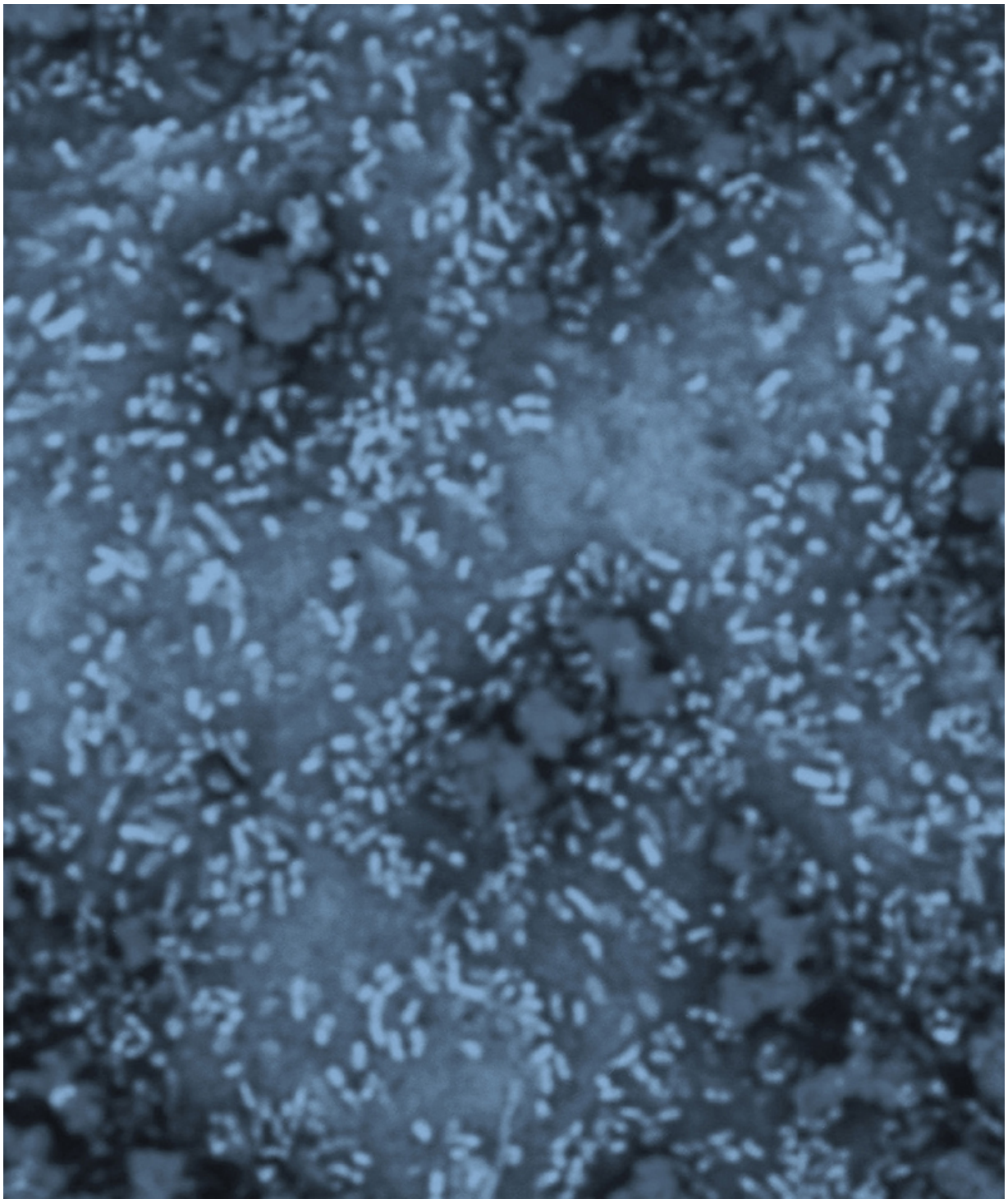


Paenibacillus spp.

TEAMING WITH BACTERIA

The Organic Gardener's Guide to Endophytic Bacteria and the Rhizophagy Cycle

JEFF LOWENFELS

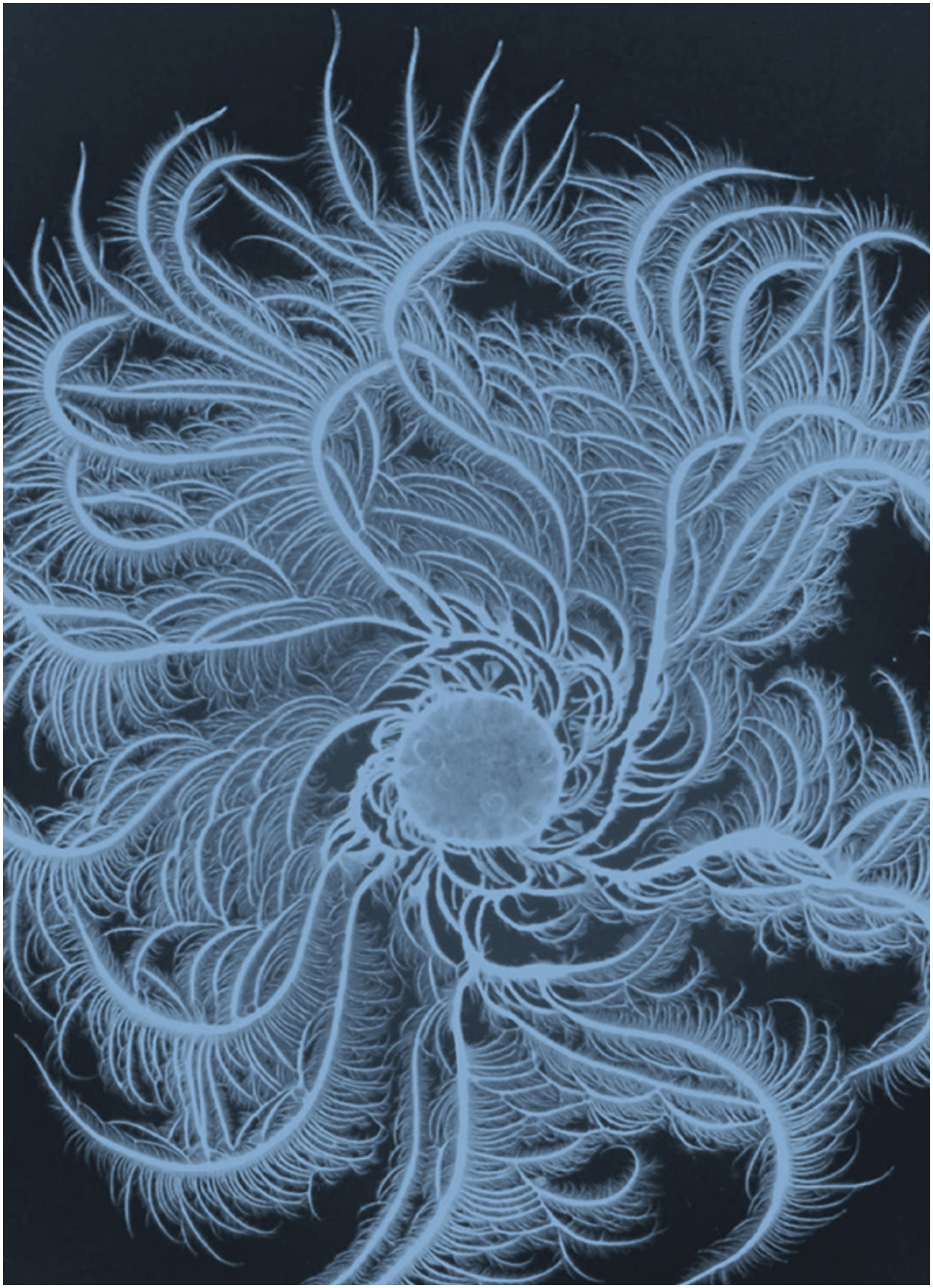
TIMBER PRESS
Portland, Oregon

For Jude

I feel it in my branches, I feel it in my roots
Endophytes are in me, from my flowers to my roots.
—from Andy MacKinnon's endophyte
sing-along at a Washington Native Plant
Society meeting

CONTENTS

Foreword
Preface
Botany for Students of Endophytes
Biochemistry for Students of Endophytes
Bacterial Review
Endophytic Bacteria
The Rhizophagy Cycle
Growing with Endophytic Bacteria
The Endophytic Future
Resources
Further Reading
Photo and Illustration Credits
Index



FOREWORD

PLANTS EVOLVED IN the presence of bacteria and other microbes; they could rely on microbes through evolutionary time for nutrient and development control. Rhizophagy delivers nutrients to plants, but that is not the only function of the microbes involved. Plants also use intracellular microbes in their development and growth processes. The bottom line is this: without microbes (mostly bacteria), plants get less nutrients and cannot develop properly. Those are two facts.

It is not only about rhizophagy (a cycle that definitely delivers nutrients, as multiple experiments have shown) but also development (again, per multiple experiments) and other effects that we do not yet know—for example, all the effects intracellular microbes have on plants and plant gene expression.

Healthy plants really are "teaming with bacteria." It is mind-blowing, but the bacteria are distributed all over the plant—shoots, leaves, and fruits. Where plants do not oxidize them, these endophytic bacteria even enter into nuclei. We have seen them in chloroplasts in some cases—probably to escape being degraded in the cytoplasm.

I had a visit with a creative young scientist, David Johnston-Monje (who authored an article about endophytes in corn) in Cali, Colombia. Over some craft beers we discussed recent observations concerning microbes emerging from tomato glandular trichomes on leaves and stems. We speculated (hypothesized) that all plant hairs could originate from intracellular bacteria stimulation—and that the function of this emergence of microbes from trichomes onto the surfaces of leaves was to originate the phylloplane microbial community for defense of leaves.

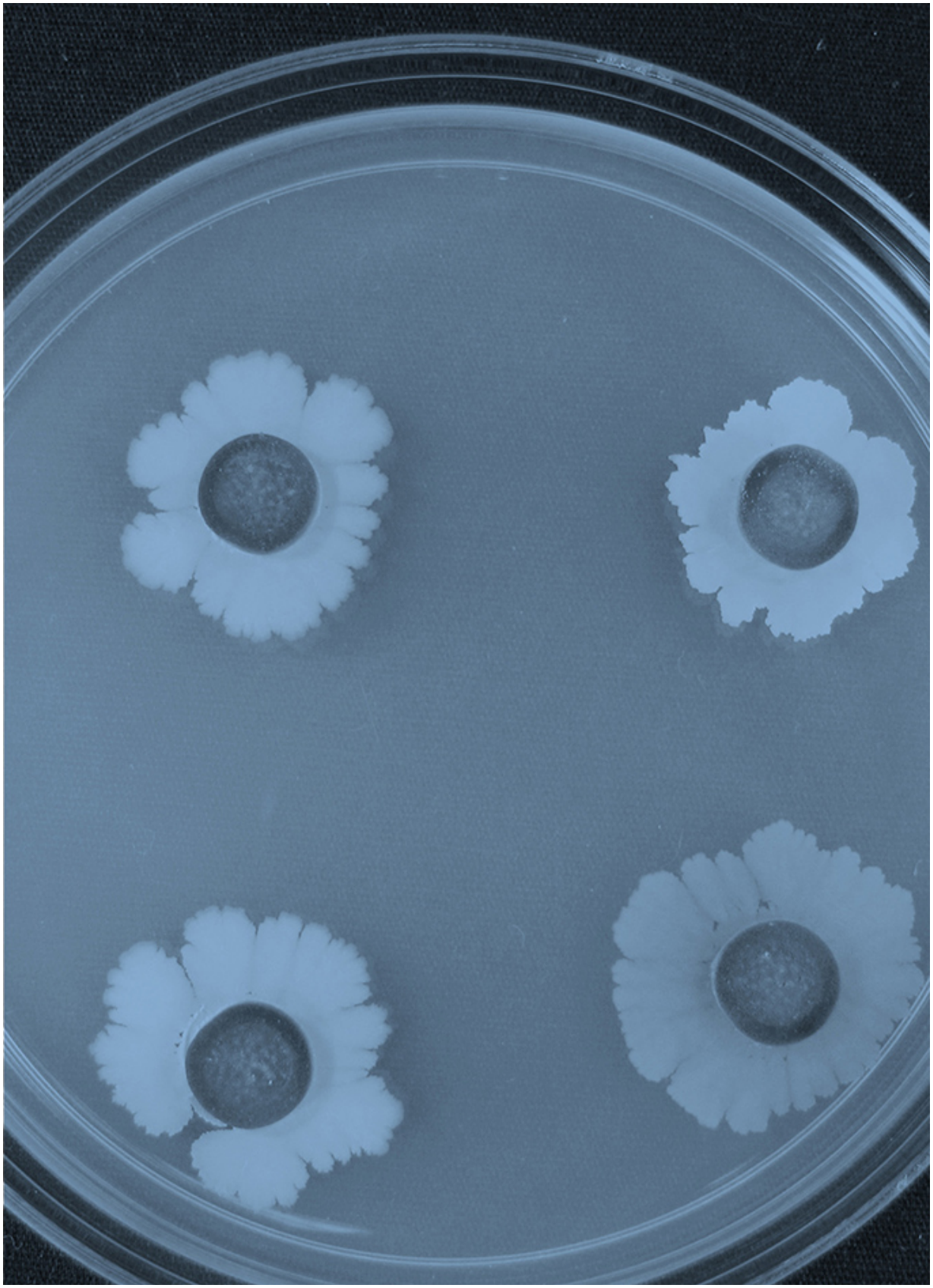
We know that cannabis makes lots of glandular trichomes on flowers and fruits. Are microbes important in the development of those cannabinoid-laden structures? Do the microbes affect cannabinoid content? We don't know.

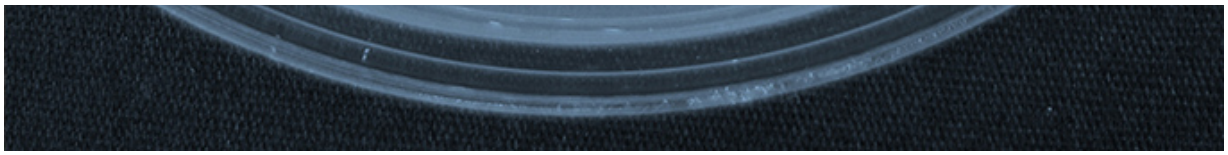
I think this book is important and will give people a glimpse into a future where most of what we thought about plants will be modified substantially.

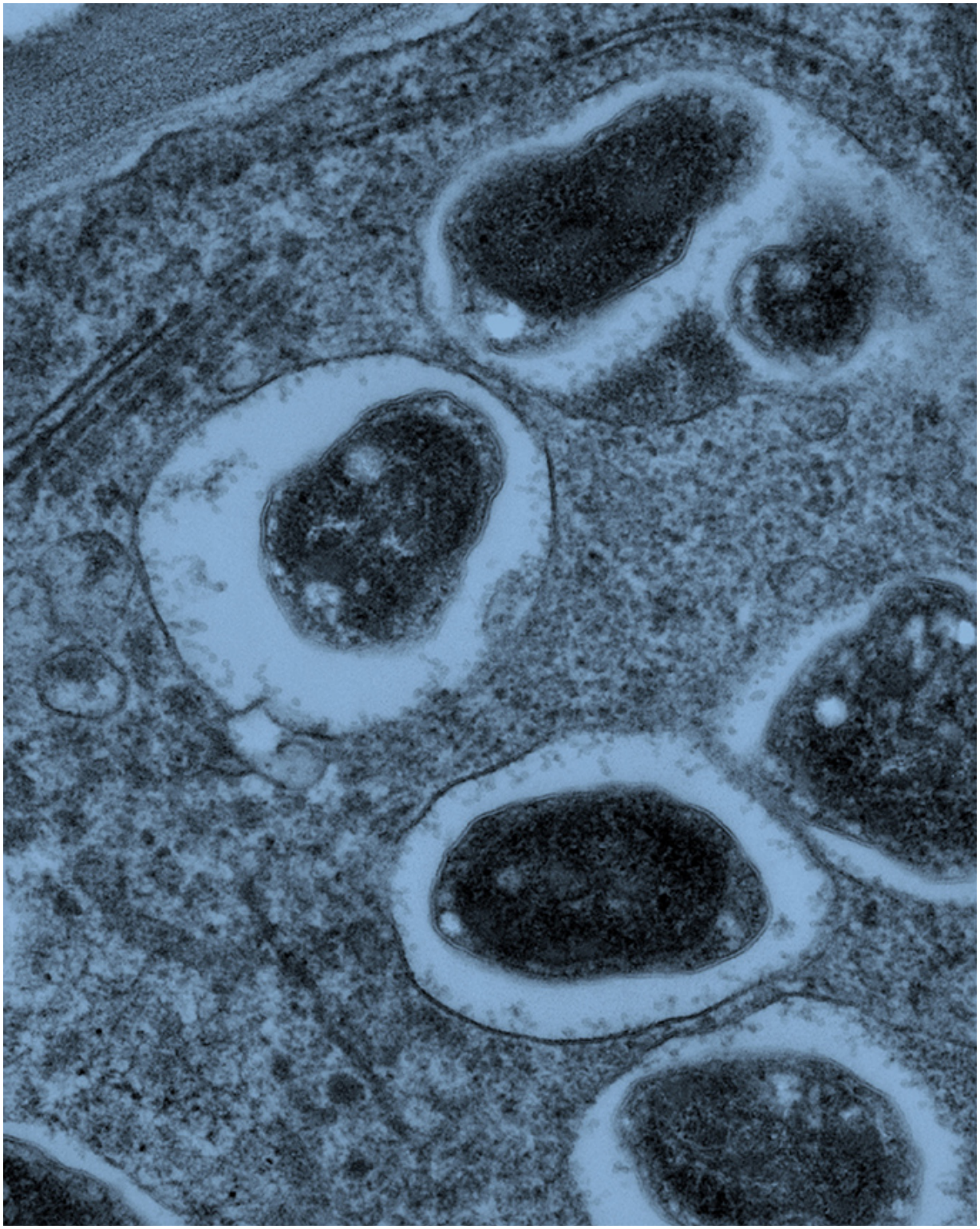
The plant microbiome will play a much more prominent role in thinking about plants.

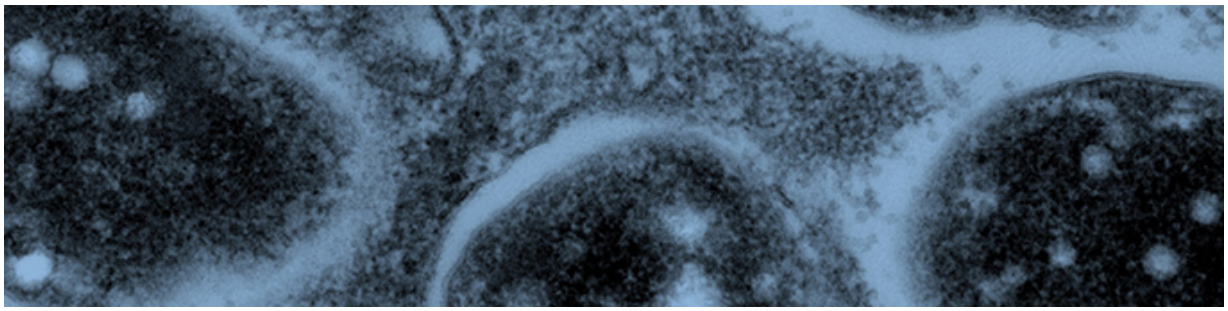
Some of this book's young readers may be the ones to make new discoveries about how the plant microbiome works. I hope that will be the case.

James Francis White
Rutgers University
March 2021









PREFACE

IT IS DIFFICULT to write a book—truly not a task to be undertaken lightly. To do it justice, you need to crawl down a rabbit hole and stay there until the job is done. It is lonely down there, so the subject matter has to be worth it. Having reemerged, I can say with confidence: rhizophagy is most definitely worth the trip.

I first saw the word in 2010 when my friend Ross sent me a one-word text: "Rhizophagy!" At first I thought he was calling me a weird name, but I knew the word's Greek roots (*rhizo*, "root"; *phagy*, "to eat"). So—rhizophagy, root-eating. No other information was available back then. I eventually learned that rhizophagy was root cells engulfing microbes and using them for nutrients. I filed it away.

Fast forward ten years. While reading up on endophytic bacteria—those that live inside plants for part of their lives without causing the plant harm—I came across research papers on the subject of rhizophagy by James Francis White, a professor at Rutgers University. His team confirmed and expanded earlier studies conducted by Chanyarat Paungfoo-Lonhienne and her associates at the University of Queensland, Australia. These scientists deserve our attention. Nobel Prizes for you all.

Endophytic bacteria are found inside every single plant. They usually help the plant, by aiding nutrient acquisition, increasing stress tolerances, and controlling pathogens. Yet little is written about endophytic bacteria for the gardening world. What's more, it appears a new plant—microbe nutrient cycle is in play. Bacteria move from the soil into young root cells at root tips. As they do, they trigger the plant to produce chemicals that dissolve the bacteria's cell walls. Amazingly, the wall-less bacteria remain viable and actually increase in number as they are circulated around the outer edges of the root cell—and the dissolved components of the bacterial cell walls become plant nutrients.

In an attempt to neutralize the wall-removing chemicals, the intracellular bacteria produce nitric oxide; this is converted to nitrates, and these too are absorbed by the plant root cell (more nutrients!). And all the while, these

bacteria are releasing ethylene into the plant cell, causing the cell to elongate.

When enough of these wall-less bacteria accumulate in the root cell, their ethylene causes a root hair to form, and they are ejected from its tip back out into soil. The formerly endophytic bacteria, now living in soil, rebuild their walls, fatten up, and repeat the cycle.

BREAKING NEWS

Wait. What? Which plants? All plants that have root hairs. Root hairs are formed by intracellular bacteria? And these intracellular bacteria provide significant amounts of a plant's nutrient needs? This is in addition to the nutrients derived from the soil? Time for another book.

I thought long and hard about whether another trip down the writer's rabbit hole would be worth it. When I emerged the first time, I had a manuscript for *Teaming with Microbes: The Organic Gardener's Guide to the Soil Food Web* (Timber Press, 2006). I made that journey because I was as excited then as I am now about bacterial endophytes and the rhizophagy cycle. I knew other gardeners would benefit from knowledge of the soil food web, a cycle which had been uncovered primarily by Elaine Ingham but was not yet generally accepted. If there was an introductory book on the subject, other people might get excited about Dr. Ingham's work, and I would have gardeners to talk to about microbes, soil, and plants.

Teaming with Microbes (you do need to read it) is all about the soil food web as we knew it at the time. At its core is this premise: plants are in control of soil microbes, which they attract to their root zones. These microbes produce nutrients in inorganic form so they can taken up by the plant.

The second time I went down the hole was to explain how plants used those nutrients. *Teaming with Nutrients* (Timber Press, 2013) explains how plants take in the nutrients the soil food web makes available to them and what happens to the nutrients once inside the plant.

I never planned to go down the rabbit hole again, but fungi (and a type of endophytic fungi at that) are a huge part of the story of how microbes make food available in the soil. *Teaming with Fungi* (Timber Press, 2017) was that trip's result. A trilogy of *Teaming* books and I was done.

Ah, but who knew, then, that endophytic bacteria were helping plants grow? And who knew about the rhizophagy cycle? So, a fourth trip—

resulting in this book on the benefits of endophytic bacteria, complete with an introduction to the amazing rhizophagy cycle. The activities of these special bacteria add to our understanding of the soil food web concept of microbes feeding plants. It's too early to say our understanding is complete. But some of these bacteria are already on the market. Professional growers use them. Gardeners should as well.

BACTERIA INSIDE PLANTS

Where have endophytic bacteria been all these years? I remember learning Louis Pasteur pulled some juice from a grape with a sterile pipette and couldn't get it to ferment. This caused him to assert, wrongly, that it was sterile inside the grape and, ergo, inside all plant parts. (Really? Didn't anyone think to ask him: Louey, how come fruit rots?) In any case, today we have pictures, and these pictures don't lie. Intracellular bacteria occur all throughout plants.

So why haven't gardeners realized that their plants are full of beneficial bacteria? Well, bacteria are really, really small, so small we have trouble envisioning them even in our most imaginative mind's eye. The tip of the tiniest pin carries thousands of them, lined up like bricks in a wall. Ever see a flea? I haven't either, but a flea is covered with loads of bacteria; the flea is huge compared to them.

Since no one was really looking for bacteria inside plant cells, they usually went unseen. Well, the type of microscope makes a difference too, but when they were first glimpsed (somehow), they were classified as Golgi bodies and vesicles. The pictures look like some of Dr. White's Rutgers' photo shots, hairs full of what we now know are bacteria. They saw them in the '90s, but they just didn't know what they were looking at.

Researchers who study endophytic bacteria had to develop special techniques to prove they were inside plant cells. They employed special microscopes and cameras. They used stains to detect bacteria and their metabolites. They even found ways to record defensive compounds used by plants in conjunction with these bacteria. It was all very complicated (and still is), but what discoveries! Plants not only farm bacteria in the soil, but they also herd them into cells and strip off their coats before returning them to the soil.

EXPANDING THE SOIL FOOD WEB

It may not be well known now, but the rhizophagy cycle represents another way plants obtain nutrients, one that is very different from soil-supplied meals. Here, bacteria nourish the plant from inside its root cells. They enter the plant, feed it, and then move back into soil. It is a big addition to how the soil food web operates.

Rhizophagy harkens back to the 18th century, when scientists were convinced that plant roots ate soil to get nutrients. This is why Jethro Tull, an English solicitor and agriculturist, persuaded farmers to rototill, a practice we now know does harm to the soil food web. This Humus Theory was ditched once German chemist Justus von Liebig invented “artificial manures,” precursors to modern chemical fertilizers. Proponents of the Humus Theory would view the rhizophagy cycle as vindication that they were right after all. They would have to have waited until 2008, however, which was when the rhizophagy cycle was first reported. It was hard enough convincing growers that microbes had a real role back then. I am hopeful now that it will be easier to accept that plant roots do, indeed, eat microbes!

STAY TUNED

This is only the preface to the story, so I won’t spoil it. But contrary to what you learned in grade school science class, root hairs are not just for adsorption anymore. It appears every plant that produces root hairs engages in the rhizophagy cycle.

There is much to learn about endophytes in general and the rhizophagy cycle in particular. Make no mistake, the story I tell here is not mine but that of a handful of very, very dedicated, smart (and equally excited) scientists who just haven’t had time (or perhaps the spousal permission, for which I am so grateful) to go down into a rabbit hole to write it up for the general public. A growing cadre of cohorts around the globe continue to make astonishing discoveries, on a weekly basis, that will surely and swiftly make parts of this book obsolete. Still, we now have a model with which to start understanding an important, additional way plants obtain nutrients. This is exactly what we need if we are to feed a rapidly burgeoning world population and deal effectively with climate change and soil loss.

Gardeners, farmers, and other growers: we need to make sure our best growing practices take endophytic bacteria into consideration. They can make plants grow better, naturally. We must adjust best practices (and develop new ones) to ensure the rhizophagy cycle (and indeed, the whole soil food web) can operate most efficiently and that the right endophytic bacteria can do what they are supposed to do without interference.

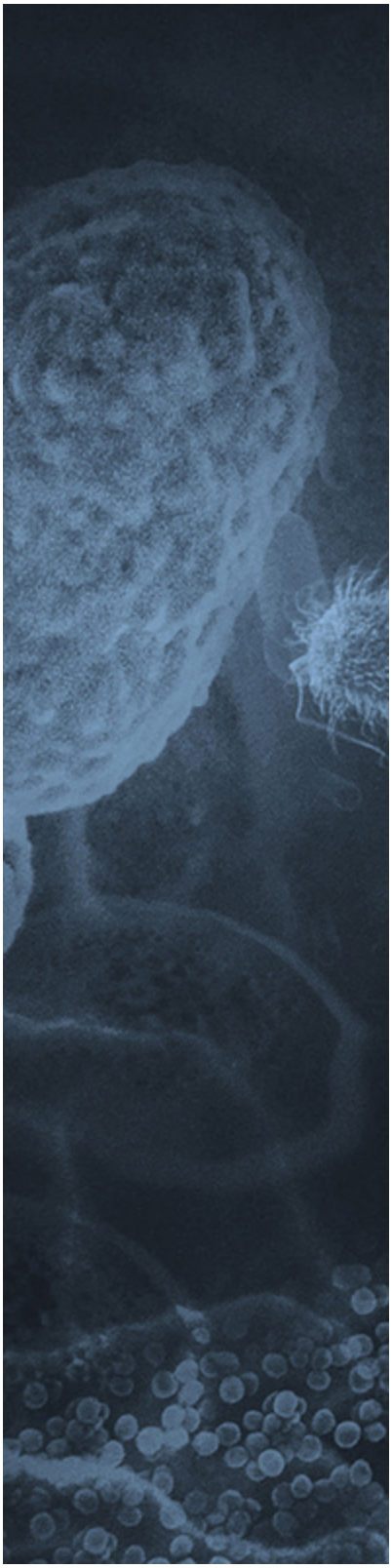
CAVEATS

A few caveats are necessary before we begin. We need to learn some science to understand endophytes and how to work with them. In the pages that follow, I have tried to limit the scientific information (botanical, biological, biochemical) to what you absolutely need to know so you can have a running start at understanding endophytic bacteria and the rhizophagy cycle. I repeat (maybe too often) the important stuff so the science should diffuse into you (like nitrogen into a plant). In any case, there is no test. Relax.

Next, don't feel you have to memorize anything as you read this book. And this goes, in particular, for the scientific names of bacteria, from phyla and genera to species and strains. You will initially be a bit freaked out (I was), but I assure you: you don't even have to pronounce them to yourself as you read, much less memorize them. The important ones will be repeated often enough that you will become familiar with them by sight, and that is good enough for now.

Third, I am crazy about gardening and love science, but I like to joke around too. Forgive me in advance for my attempts at humor, earlier and to come. (I usually add these comments in parentheses, to help you identify what you might want to gloss over.) I also ask a lot of questions in this book, and I sometimes speculate on the answers. (I believe speculation stimulates thinking.) I hope these occurrences are obvious.

Finally, I supply a few thoughts about the future of gardening, growing, and farming with endophytes. (I warn you, I am opinionated—just ask my family and friends.) It may seem like speculation, but I have a track record. The soil food web and mycorrhizal fungi are now mainstream. I am certain that endophytes and the rhizophagy cycle will be too.



Botany for Students of Endophytes

AS A GARDENER, you don't need to know anything about, say, the location of particular plant cells to successfully germinate a seed or transplant a start—but some knowledge of how plants operate is required before you can appreciate endophytic bacteria and the rhizophagy cycle. This, in turn, requires a measure of familiarity with specialized language that many of us simply don't know (or did know at one time but have now forgotten).

And so we start with a review of some basic botany—promise, it will be painless. You may wish to review the other books in the *Teaming* series, and it never hurts to have a whole book on botany on your reference shelf. My favorite is the fourth edition of *Botany for Gardeners* (Timber Press, 2022).

SEEDS

Let's begin with seeds. Why? Endophytic microbes may enter plant roots via the soil, but seeds are often how these bacteria get into the soil in the first place. Seeds contain bacteria necessary for seedlings to get a good start, and these bacteria either remain in the plant or move into the soil. I am referring here to seeds of flowering plants, angiosperms, as that is what we plant in our gardens and on our farms. Gymnosperms ("naked seeds") don't employ the same seed structures, but they too host endophytic bacteria and their roots participate in the rhizophagy cycle.

If anything about growing a plant is taken for granted, it is the seed. You stick one into soil, give it water, and off it grows. All the gardener needs to know is whether the seed is viable (which might have to do with its bacteria, actually), how deep to plant it, and how long it will take to germinate. Not much time is spent contemplating the wonder of seeds—what they do and how they do it; we are much more interested in the process's end result.

Seeds of particular species tend to have the same bacteria in and around them from one generation to the next. This is because very specific endophytic bacteria are carried in their seeds. And there are even different

strains of bacteria for different strains of the same plant species; these endophytes may be why your heirloom tomato differs from mine.

Seeds are the containers that hold the plant's DNA. Each seed contains a miniature plant as well as the food necessary to sustain this embryo until it can produce its own supply. The seed's bacteria surround the embryo but are not found in it (perhaps because they might decay it before it can germinate).

SEED COAT

Consider, then, the structure of a "typical" seed. Designed by evolution to facilitate survival after dispersal (and sometimes even involved in its own dispersal), a seed has three primary parts: a hard outer coat, a tender embryo, and embryonic leaves. As a gardener, you've undoubtedly encountered all three. The seed coat not only protects the embryo from being squashed, it keeps it from drying out. This requires a relatively hard wrapper (think box) with some special properties.

The seed coat consists of layers of cells known as integuments. Integuments surround ovules, the part of flowers in which seeds form. A flower's integument becomes the tegmen (a great crossword puzzle word), the seed coat's inner layer. A second integument becomes the outer (sometimes only) layer, the testa. In monocot seeds, the tegmen and testa merge, growing together. Testa cells often produce astringent compounds that help protect the seed from consumption by predators before it is ripe; these tannins are what cause the dry, sour taste of unripe fruit.

The seed coat protects the embryonic tissue passed on from the mother plant. Embryos can't develop without water and oxygen. Seed coats are mostly impermeable by water but selective as to gases. Some testa have an opening, the strophiolar cleft. (Scientists have a word for everything.) It is plugged. Removal of the plug enables water and gases to enter the seed, which triggers germination. Plants that don't have a cleft rely on abrasion or chemical degradation (such as when a seed goes through a mammal or freeze and thaw cycles) to allow water to enter. Not surprisingly, some seed coats are degraded by microbes, and this lets in the water.

As the primary root grows during germination, it is protected by a sheath, a coleorhiza. The shoot, on the other end, is also protected by a sheath, the coleoptile, which stops developing when the plant is exposed to light, causing the embryonic leaves to unfold. Meanwhile, bacteria that are

in the seed develop populations in the soil and in the plant as it germinates and grows.

THE EMBRYO

The embryo carries with it all the DNA for a new plant. All embryos consist of three parts: a radicle, the hypocotyl, and cotyledon(s). Let's start with the radicle. These are the cells that will develop into the primary root, the taproot that grows downward into the soil. Side or lateral roots grow from it.

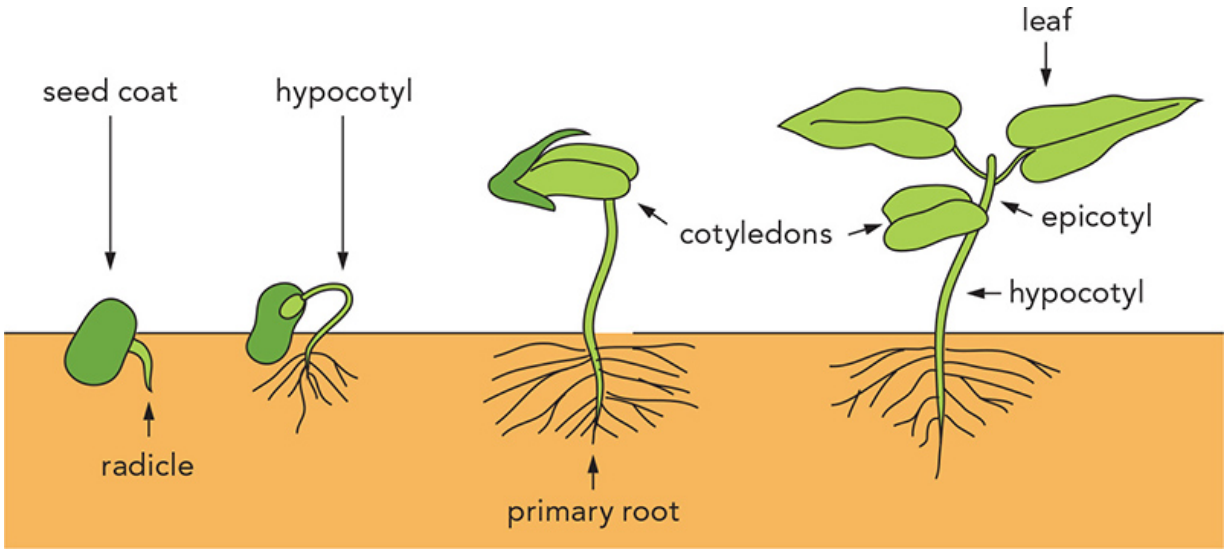
Next is the hypocotyl, the embryonic shoot located between the radicle and the cotyledons. It becomes hooked-shaped immediately after germination, which protects the fragile new seedling as it grows through soil. When exposed to light, the hypocotyl straightens out, elongates, and points the leaves toward the sun.

Cotyledons are the embryonic leaves, sometimes referred to as seed leaves. These open after germination and provide the photosynthetic goodies to the seedling (whose stem, the epicotyl, is located just above the cotyledons). If the seed has only one cotyledon, it is a monocotyledon, a grass. Gymnosperms have at least two (and often more) cotyledons.

GERMINATION

Seeds can live a long time before germinating (a 2,000-year-old date palm seed may hold the record). How is the embryo able to survive for so long? It is either embedded in nutrients to use while inside the seed or it has evolved to survive without nutrients, so long as there are some to assist at the start of germination. There isn't usually much biological activity inside a seed anyhow, so a little bit of food goes a long way.

Either way, seeds have a very limited amount of nutrient support beyond what they require for germination—about a two-day supply, on average. While inside the seed, proteins used later for support are suppressed from developing into anything except food. Given the limited material and energy at hand, this makes perfect sense. When conditions are right and growth is triggered, proteins are used in the biosynthesis of needed parts and the plant grows. In case you are wondering, gibberellic acid (my favorite hormone) is the regulator that speeds up seed germination.



A seed germinates into a seedling.

A SEED'S MICROBIOME

The first of the two basic groups of seed microbes are the endophytic microbes; these reside inside the plant. The second group, epiphytic microbes, live on the surface. Some bacteria have dual roles, converting from one group to another.

Endophytic bacteria are most often transferred vertically. They start out in a seed, move into the plant, grow inside its flowers, and move on to the next plant via seed. Or they can enter a plant horizontally, via a wound, for example, or swimming into a leaf's stomata. It is even possible some insects, pollinating or otherwise, bring them in. Epiphytic bacteria are usually transferred from plant to plant horizontally, traveling in the wind or, again, on the wings and legs of insects.

Different parts of the seed contain different microbes. The coat can have one microbiome, with epiphytic microbes on the surface and endophytic inside the surface. The endosperm, those stored nutrients in seeds, will support completely different kinds of endophytic microbes. Some of these microbes are transient, moving to the soil where they potentially impact the next generation of seeds. One thing is for sure: bacteria are everywhere.

The choice of which bacteria a particular plant species associates with appears to be different for each plant genotype (unique genetic composition). When left to Nature, seed types pass the same groups of bacteria from one generation to the next, carrying on strains that are similar to those of their ancient relatives. Of course, a seed's microbiome is also

influenced by climate, the presence of other microbes, plant breeding, and other agricultural practices. Many of our crop seeds no longer carry the microbes their ancestors did.

One of the tenets of the soil food web is that the plant chooses the bacteria it needs and not the other way around. A recent study showed how a tropical legume tree can even go so far as to change the soil's pH to attract iron-fixing bacteria so it doesn't run out of needed nutrients. (Amazing.) There simply is no question: plants are in control when we let them be—not only of the soil bacteria they attract but of who gets to move into them, from the soil.

In short, endophytic bacteria are plant-specific, and specific ones reside inside seeds. Consider that the plant has to evolve to allow bacteria inside its cells. It is a big deal to let down cell walls and defenses against bacteria. Mycorrhizal fungi invade only in between cells, so the host's defenses may not be fully activated (as they would be if the invasion was intracellular). This may be why the same fungus can associate with many different kinds of plants, but a bacterium cannot: the plant has to be much more careful in distinguishing between good and bad bacteria.

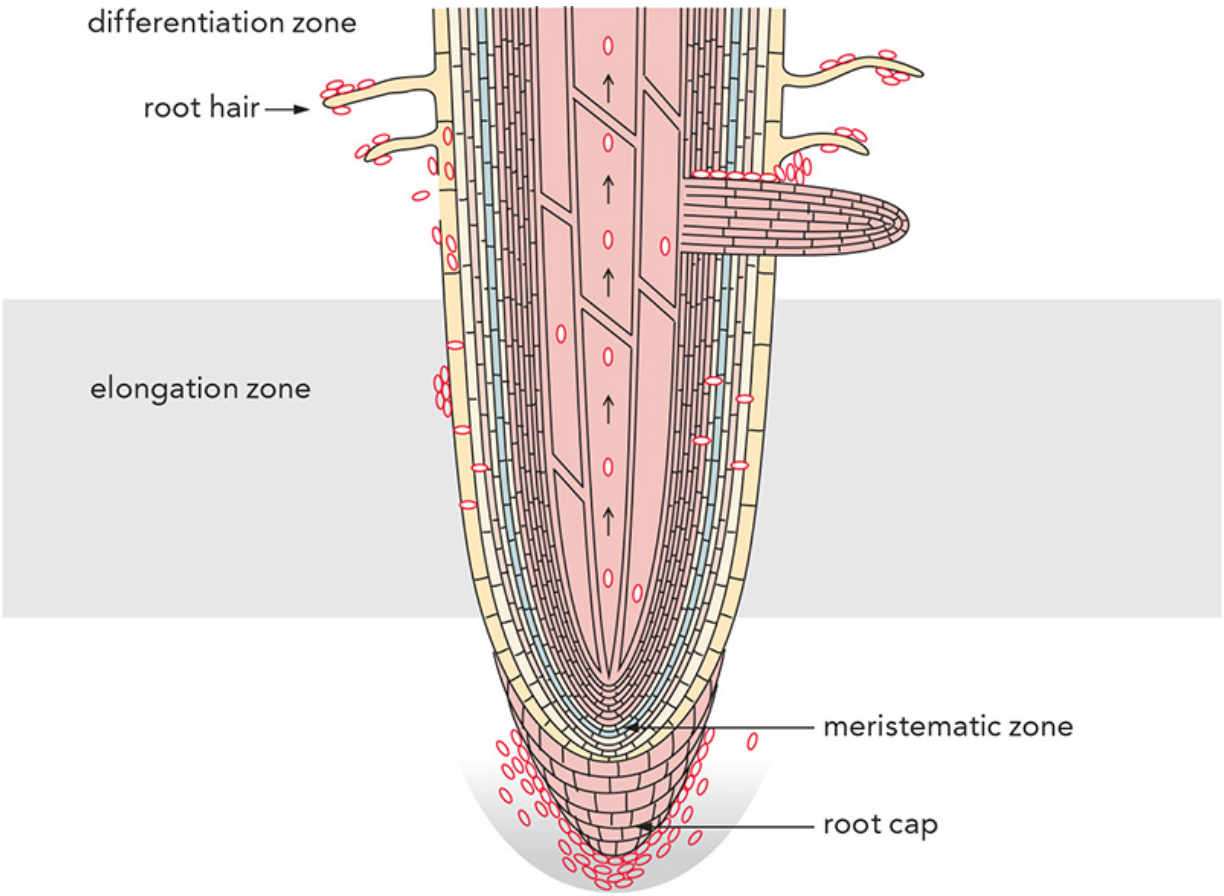
Finally, bacteria in a seed's microbiome are highly diverse, with as many as 9,000 different microbial species represented. Certain bacteria phyla that dominate in the soil are found prominently in seeds: Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria (that wasn't so difficult). These groups provide a candidate pool for the endophytic and epiphytic bacteria that live on or in plants.

A TYPICAL ROOT

Endophytic bacteria can locate in all parts of a plant, but the rhizophagy cycle takes place in the roots. This is why it is necessary to examine the root just a bit, even though as gardeners and growers, we know the importance of roots. Roots anchor the plant to the soil, are responsible for absorption and nutrient uptake, and help orient the plant. Now we need to add that roots are the exclusive hosts of the rhizophagy cycle.

There is no typical, universal root, but I am going to pretend there is one. This typical root can be divided into four parts. At its very tip is a protective root cap. This is followed by an area of cell division, the apical meristem. Just above this growing tip is an area of cell elongation, which provides the

force that moves a root through the soil. The last zone is where cells finish development, a zone of differentiation where maturation occurs.



Typical root zones.

ROOT CAP

The function of the root cap (aka calyptra) is to protect the tip of the radicle. The root cap acts just like the heat shield on a space capsule. The tender, thin-walled and fragile meristem cells immediately behind the root cap are protected as the root grows. Soil is abrasive, and so these cap cells are short-lived: they need to be replaced continuously.

The old root cap cells (aka border cells) slough off into the soil. They are biologically disassembled (good term for being decayed) by the microbial herd in the rhizosphere, the area right up against roots. Sometimes they are chemically dissolved before bacteria and fungi can get to them. Either way, root cap cells are a part of the exudate nutrient pool plants put into the soil.

Root caps contain statocysts, starch-filled structures that act like weights and drop to the lowest part of the cell, causing the characteristic downward growth of roots. They help the plant sense gravity. When they are removed, roots grow in random directions. (Spoiler alert: the same thing happens when the endophytic bacteria involved in the rhizophagy cycle are removed.)

Another player in the root cap area is mucilage, which lubricates the soil through which the cap moves. Like putting soap on a screw (try it), this slippery, slimy liquid means the entire root has an easier time moving into and through the soil. Mucilage also helps keep the root from drying out and acts as a glue to help attach soil and microbes to the root's surface.

As root mucilage oozes into soil pores, its chemical composition and pH attract phosphorus, zinc, iron, and magnesium. These metals then diffuse into the root as nutrients. Mucilage also serves as a microbial attractant. No doubt at least some of the bacteria that participate in the rhizophagy cycle utilize mucilage as food before they begin their journeys. Who knows (we will soon): mucilage might even be the attractant that brings rhizophagy cycle microbes to the root's surface in the first place.

MERISTEMATIC ZONE

The cells that are protected by and located immediately behind the root cap are totally undifferentiated. These are apical meristem cells. They are all the same, each one capable of becoming any part of the plant. What part is entirely up to the needs of the plant. A signal to the appropriate meristem area (there's one at the tip of the shoot too) causes an undifferentiated cell to specialize and mature. Once an assignment is received, a meristem cell has its purpose and can no longer mature into anything else.

While normally the shoot meristem produces aboveground parts (stems, leaves, and flowers), it is possible for a plant to signal a root meristem cell and cause it to move and develop into an aboveground organ.

Meristem cells are packed together tightly. They have very thin cell walls, which makes them easier for bacteria to breach. They also have large nuclei. Meristem cells divide every 12 to 36 hours. Further back in the older part of the root, a cell can take 200 to 500 hours to divide, and their nuclei are smaller. Plant cell division, incidentally, is rhythmic, with peaks at noon and midnight. Who knew?

Some plant roots add meristem cells laterally in addition to those in the main root; this increases the diameter of the root and provides cells that can develop into side branches.

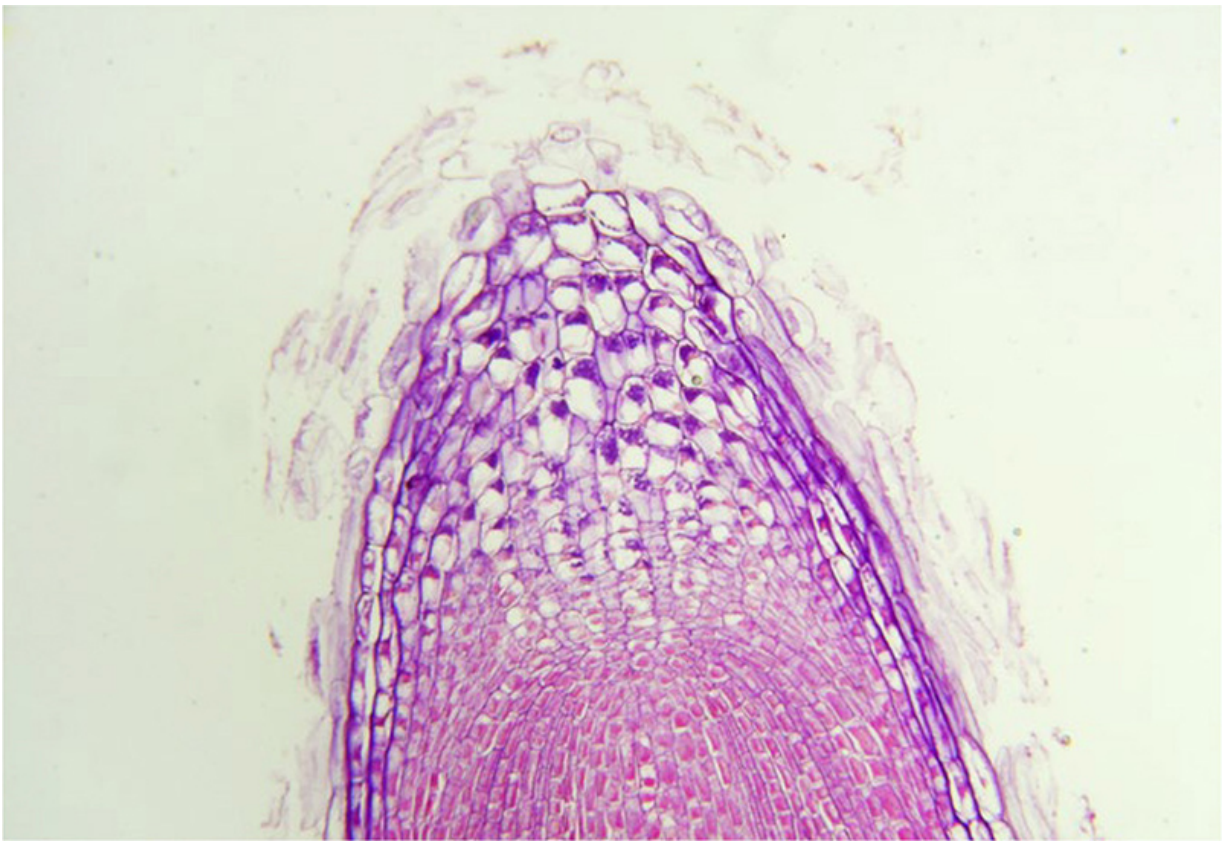
Bacteria produce ethylene. Plants produce it as well. When bacteria amass inside a meristem cell, they can get caught up in a bulge in the wall. Enough ethylene accumulates, and the meristem cell begins to grow a root hair (but we will get to that).

ELONGATION ZONE

Meristem cells increase in length several times in the elongation zone. The energy for elongating cells comes from small vacuoles (storage bubbles) inside the meristem cells; these fill with liquid, merge, and get bigger as the cell grows. Here is the source of the tremendous hydraulic pressure that drives the root into the ground. Ethylene gas triggers and modulates root cells to elongate. At least when it comes to root hairs, rhizophagy bacteria appear to be the source of this elongating ethylene.

DIFFERENTIATION ZONE

The differentiation zone is where meristem cells mature and become fully developed cells with assigned roles. A cross section of this area reveals the root's outer, single-cell epidermis surrounding a cortex consisting of several layers of cells: xylem tubes (for water), phloem tubes (for sugars), a single-cell endodermis, and in the center, the pericycle. We are chiefly interested in the epidermis—the area where root hairs form.



A root cap magnified. Can you see mucilage, border cells, meristem cells, and elongating cells?

ROOT HAIRS

Root hairs are special epidermis cells, or better put, part of a special epidermal cell; they are extension tubes that form in the cell's wall. The hair's tubular shape is caused by the pressure building from within and the restrictions of the cell wall. The new wall at the tip is flexible as it extends. The side wall is rigid. If you were inside a cell with a root hair growing, you would be able to swim around: there is no wall separating the root hair from the cell.

Clearly, root hairs play a key role in developing and sustaining their plant. They are parts of young, very thin-walled cells that do not yet have fat molecules (which would repel nutrients rather than absorbing them). Root hairs also increase the surface area of these thin-walled cells, doubling (on average) the surface area of roots. This, obviously, allows greater uptake of both water and nutrients.

Add to the tiny root hair's absorptive character its ability to grow between soil pores; there it is able to obtain water and nutrients that would

otherwise be inaccessible to the considerably larger root (though mycorrhizal fungi might help). Root hairs are not long-lived, functioning for one to four days. The older hairs die at the back end of the differentiation zone. At the same time new hairs are constantly being formed at the start of it.

Here is the big rub: root hair elongation is a direct result of endophytic bacteria that participate in the rhizophagy cycle. There is a bit of chemistry involved in this, and we have not covered what we need in that area yet. Suffice to know endophytic bacteria are involved in root hair formation. We need to add a new purpose for root hairs: to thrust rhizophagy bacteria back into new soil. And note: this bacterial placement mechanism may be the primary structural purpose for root hairs, not the absorption of water and nutrients. Actually, it is probably both. Stay tuned.

EXUDATES ARE KEY

A word or two about plant exudates, which are key to rhizophagy and endophytic bacteria. Exudates are water-soluble organic substances produced by the plant and put into the soil; they are important because they attract and support soil microbes. Plants modulate their microbial populations by altering the quantities and qualities of their exudate mix. It takes a tremendous expenditure of energy for a plant to produce exudates.

Exudates are mainly mixes of sugars, amino acids, organic acids, and enzymes. In attracting bacteria and fungi, they are joined by border cells sloughed off as the root tip moves into new soil and by the mucilage lubricants, as well as by any parts of the root system that break off and remain in the rhizosphere. But it is exudates that act as primary movers when it comes to attracting and supporting fungal and bacterial populations.

At least one exudate, an amino acid, plays a key role in the rhizophagy cycle. That exudate is L-arginine, often just arginine. It is necessary for the process bacteria use to make the ethylene, which triggers increases in plant cell growth. More on this important exudate in the next chapter.

Root hairs, all with (now) visible bacteria.

CYCLOSIS

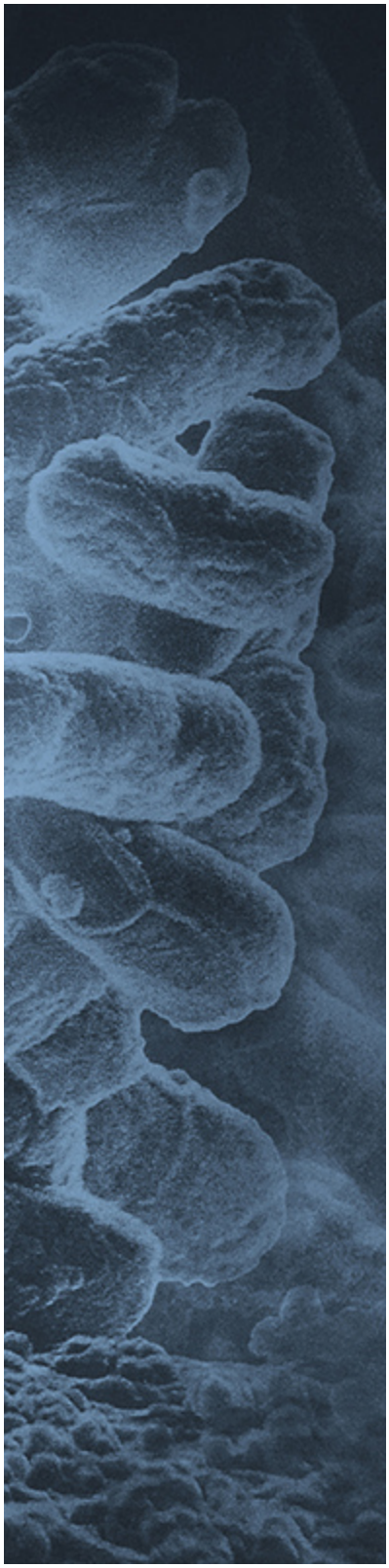
Cyclosis is the movement of cytoplasm inside a cell. As the name of the process suggests, the motion is a cyclical. It is also uni-directional, though there are all manner of eddies and currents in the flow (think cytoplasm flowing around molecules as a river flows around and over rocks). In plants and animals, this cytoplasmic (or protoplasmic) streaming is caused by the scaffolding infrastructure (actin fibers, tubules, and the like) inside plant and animal cells along which materials and signals move. As they do, currents are created in the liquids in which they float. Bacteria don’t have the same cytoskeleton, nor do they have streaming.

What is important is that things move around inside the cell. These might be synthesizing or nutrient molecules, enzymes in action, or specialized chemicals. Chloroplasts, for example, are moved around plant cells via this circular flow, in part, to better orient to the sun.

And, of course, intercellular bacteria themselves are caught up in a plant cell's cytoplasmic streaming. It is this circular movement in the root meristem cell, in conjunction with the production of reactive chemicals, that results in the breakup of the cell walls of participating bacteria, as they bump and rub between the cell wall and the cell's membrane. (How would you like to end up in a washing machine?)

CLASS DISMISSED

I hope this chapter has inspired you to learn more about the parts of plants and how they operate. Botany is painless, so it is strange how many of us who grow plants have never had a course in it. No matter. You have just graduated from the endophytic and rhizophagy bacterial botany review. You are now armed with enough botanical information to fully understand endophytes and the rhizophagy cycle; I will define anything else on the spot, as needed.



Biochemistry for Students of Endophytes

JUST AS KNOWING some botany helps us envisage where endophytic bacteria live and work, having a few chemical and biological concepts under our belt will help us understand what endophytic bacteria do and why they do it. The relevance of these concepts may not be obvious here, but they will be when we turn to endophytes and the rhizophagy cycle.

THE MOLECULES OF LIFE

All things are made up of atoms. Atoms carry electrical charges that cause them to bond with other atoms, even different kinds. Materials made up of only one kind of atom are elements (carbon, C; oxygen, O), and molecules comprising different elements are compounds (water, H_2O; carbon dioxide, CO_2).

All molecules in living organisms fall into one of four broad categories: carbohydrates, lipids, proteins, and nucleic acids. All have carbon as their base and oxygen, hydrogen, and nitrogen atoms in common, only arranged into different patterns (depending on the group and the way the particular atoms in the molecule bind to each other).

No matter their grouping, all molecules of life are organic compounds by virtue of their links to carbon atoms. Individual molecules assigned to any of the four basic groups can be linked together to form bigger molecules, polymers (biopolymers, to be exact)—for example, proteins are the building blocks of amino acids, and nucleic acids link to form DNA.

ACIDS AND BASES

What are acids? An easy definition: any compound that tastes sour when dissolved in water. Others rank a substance an acid for its ability to promote special kinds of reactions, such as freeing hydrogen from some metals (think rust). I say acids are any substances, organic or inorganic, that donate positively charged hydrogen (hydrogen ions, H+) to other molecules.

Inorganic substances form mineral acids like sulfuric acid, phosphoric acid, or nitric acid. All it takes is for a substance to be able to release a hydrogen ion when in water solution. The more hydrogen ions produced, the stronger the acid. The pH goes down.

The opposite of an acid is a base. Basic (or alkaline) substances produce hydroxyl ions (OH-). They combine with elements to form hydroxides, as in potassium hydroxide, sodium hydroxide, and calcium hydroxide. Like acids, hydroxides can be strong or weak. Strength is increased by freeing up more hydroxyl ions. The pH goes up.

Acids make life happen. Our bacteria, for example, use a process called glycolysis to generate energy. This is nothing more than using enzymes to break down sugars. During glycolysis, hydrogen ions are released, and the pH goes down. My point: acids are everywhere, and while all are important, some are at the very top of life's list.

FATTY ACIDS

Let's start with fatty acids, which are built up from lipids. Fatty acid molecules mixed with phosphorus, proteins, and carbohydrates make up the all-important, double-layered membranes that surround some cells. Fatty acids rarely appear in their standalone form. Instead they link with other molecules to form one of three kinds of esters, special chains of molecules. Unlike some other acids, which have unpleasant odors, esters smell sweet. In fact, they are responsible for the fragrance of fruits. In some bacteria, free fatty acid molecules can constitute over 20% of the total mass; this is a lot higher than in animals and plants.

PYRUVIC, BUTYRIC, AND PROPIONIC ACIDS

Pyruvic, butyric, and propionic acids are organic acids that link together to form carbohydrates or amino acids, depending on the type of linkage. Pyruvic acid is an intermediate byproduct of glycolysis, the breakdown of sugars which produces energy; it is easily converted back to a carbohydrate, can be made into fatty acids, and is yellow in color.

Ever wonder what gives rancid butter its distinctive smell and taste? That's butyric acid. Too much butyric acid, and it smells like vomit. I should know: my family was in the butter business for many generations, and I spent hours unwrapping sticks of rancid butter.

Propionic acid also has a distinct smell, that of sweaty body odor. It has high lipolytic activity, which just means it can break down fatty acids. Special bacteria, Propionibacteria, produce propionic acid. Interestingly, the holes in Swiss cheese are caused by CO_2 bubbles, which are created when those special bacteria break down the lactic acid in Swiss cheese curds. Propionic acid is important in the synthesis of vitamin B12.

These three acids have a key biological function: they act as growth regulators in plant cells. Their presence (or lack thereof) impacts the growth of bacteria and plants. Not only that, they control a part of the rhizophagy cycle.

AMINO ACIDS, PROTEINS, AND ENZYMES

Then there are the amino acids, which are built up from proteins. They control all cellular activities. More than 100 occur naturally, mostly in plants, which make all their own amino acids. Animals need to get a few from eating plants because they cannot synthesize them all.

Amino acids are organic compounds, and each one always has two special groups of molecules, the amine and carboxylic acid groups. These functional groups are what's behind each amino acid's individual traits.

Bacteria are capable of synthesizing all 20 types of amino acids that are used to make proteins. The structure of these acids determines how the protein acts. Sulfur in a chain, for example, means the protein can be circular (because of the way the bonds are arranged), and this shape would give such a protein special properties. The inclusion of other elements make for branching at different angles and so on, but we need not go that far down the well.

L-arginine is one such amino acid, a plant exudate. It is needed for plants and bacteria to produce ethylene, which is critical in helping plants adjust to changes in their environment. (This might be why its levels in roots increase during the cold winter months.) On the other hand, if there is too much arginine, bacteria populations dramatically increase; they can overpower the plant, stunting its growth. So the plant must determine how much arginine is available to the bacteria—or it would be committing suicide by bacteria.

Proteins are simply long chains of amino acids, so long they usually have to be folded; these folds are influenced by temperature and pH.

Without getting any more complicated, the amino acids are attached by bonds between the amine group's nitrogen and the carbon atom. This is a peptide bond. Two or more amino acids so linked make peptide or polypeptide chains.

Enzymes are very special proteins whose constituent amino acids are linked in a manner that allows them to function as catalysts—that is, molecules that greatly speed up but do not directly participate in chemical reactions. How much speeding up are we talking about? Enzymes increase reaction speeds anywhere from 100 million to a staggering 10 billion times. Without enzymes, chemical reactions would take hours or even days instead of mere milliseconds.

Almost all chemical reactions in a bacterial cell occur as a result of enzymes. While some enzymes have particular names (trypsin, pepsin), most are named by adding -ase to the name of the target substrate in question (peroxidase, catalase). Some enzymes are named after the reaction they create (dehydrogenase, for example).

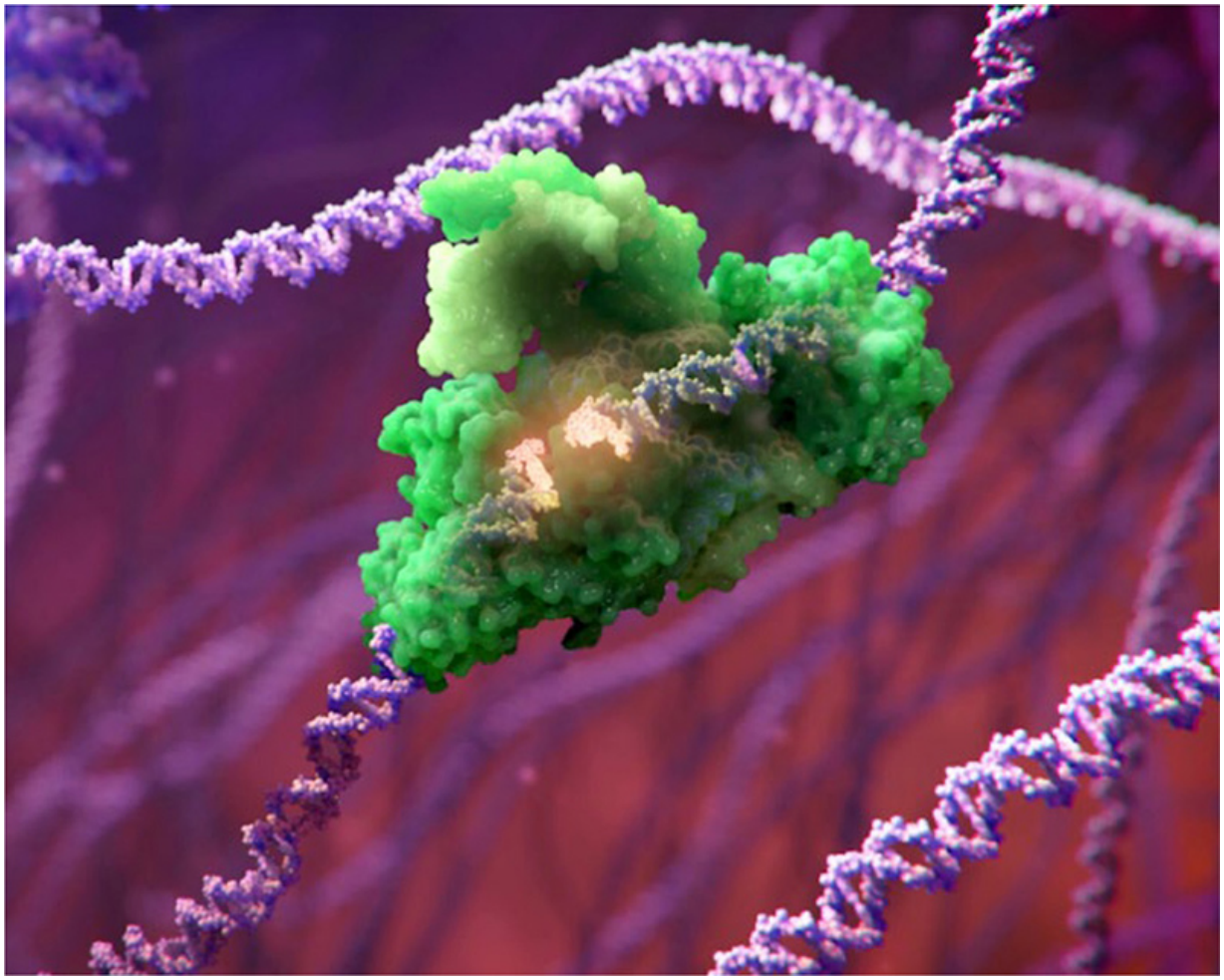
No one is exactly sure how enzymes do their jobs. The theory I learned 60 years ago is that the enzyme and the substrate act like a lock and key, their shapes fitting together naturally and precisely. This concept has been around since 1894. More modern thought, however, suggests an induced-fit theory: the enzyme and the substrate each adjust to fit together. In any case, the end result is a new product.

Enzymes are highly selective (each works only with one reaction, so you need lots of them), and they work in a range of temperatures. Those that help plants grow do best around 75°F. Some bacteria contain enzymes that work best at high temperatures, which is part of the reason they can survive.

Enzymatic activity can be slowed or even stopped by inhibitors. Some bind to the enzyme and irreversibly inactivate it. Others bind to the enzyme and the substrate at the same time, making it difficult for the end product to leave the enzyme. Still others block the active site on the enzyme (or bind someplace on it), making it difficult to impossible for the enzyme to also bind to the substrate.

Destroy any of the mechanisms that make proteins, and you cease to have life. Break any of the bonds of an amino acid or protein, and you destroy it: it ceases to function as it should. (This is what some antibiotics do.) When an enzyme is broken up, it breaks into proteins.

When a protein is hydrolyzed (that is, broken apart by reacting with water), it turns into amino acids.



DNA (purple helix) repairing an enzyme (green).

LIPIDS

Lipids are organic molecules composed of long chains of carbon and hydrogen. They are found in fats, waxes, oils, phospholipids, cholesterol, and steroids, so don’t assume they form only fatty acids. Lipids form arrays when linked together that can repel liquids: imagine a soap bubble floating in water.

In bacteria, plants, and animals, lipids are integral to the formation of membranes, the cell’s gatekeepers. Extremely tiny molecules can slip through them, but everything else has to go through a channel or tunnel to cross a membrane (in either direction).

Bacteria use lipids to store energy. Most gardeners think energy comes from carbohydrates, but lipids are much more efficient for the job. They have lots of carbon-hydrogen bonds, which are where energy is stored (and released when broken). Between the cell membrane and energy storage, lipids can make up as much as 10% of a bacterium's dry weight.

CARBOHYDRATES

Carbohydrates are polysaccharides, long chains of molecules with carbon, hydrogen, and oxygen in the general ratio of sugars, $C_6H_{12}O_6$. They store energy in their carbon-hydrogen bonds. Carbohydrates are the major source of stored energy in bacteria, even though lipids are more efficient. Usually carbohydrates are broken down to glucose, which is metabolized to produce energy.

Carbohydrates are found in the cell walls of bacteria. And just so things stick, carbohydrates are used in the biosynthesis of bacterial slime, which binds bacteria together and to soil and other surfaces, including roots.

DNA

DNA (short for deoxyribonucleic acid) is an organic compound that contains genetic code, including instructions for making proteins. It is composed of nucleotides, which units are lined up in two strands to form the familiar double helix. Nucleotides are a carbohydrate attached to a phosphate group and a nitrogen-containing base. The four bases used in DNA (A, C, G, and T for short—which is all we need) determine the specific characteristics and functions the gene will show, or in science-speak, express.

DNA not only allows a bacterium to be what it is, it allows scientists to identify what organisms are included in a mix. This is becoming easier and easier as computers do most of the work by comparing a sample with a database for matching and identification. You don't even need a single type of bacterium. When a bacteria population is involved, modern methods can identify them all.

DNA can be accidentally damaged—and purposefully altered. Bits of it can also be transferred from bacterium to bacterium without sexual conjugation; this is how bacterial species develop strains. We don't have to get too detailed here. The bottom line: the genes in DNA determine how the processes necessary for life are conducted in the cell.

GENE EXPRESSION AND REGULATION

DNA carries genes, which are responsible for specific traits. When a trait is present, it is because a gene has expressed itself. Expression occurs when a gene is activated, but the same word is used when genes are deactivated, so you have to be careful. (It's OK if you mess up as long as the bacterium doesn't get it wrong.) Some genes are always on; others are only needed occasionally and so are turned off until needed.

In bacteria, most of the regulatory proteins are designed to turn genes off, to stop them. In eukaryotes, animals, and plants, gene expression is usually off, and proteins are needed to turn genes on. This is a pretty big evolutionary difference between eukaryotes and prokaryotes.

The specific genetic information stored in genes creates a genotype. Stresses—like temperature differences or an attack by a pathogen or a mistake in the production of an enzyme—can make a bacterium different from its sister or brother even though they have the same DNA and are of the same genotype. Finally, how genes are expressed and interpreted creates a phenotype. Phenotypes can make it difficult to know who is who by mere visual observation. Only genetics can provide a certain ID.

OXIDATION

Oxidation: the word derives from oxygen, each atom of which has two unpaired electrons, which makes oxygen a great participant in chemical reactions. This is fine as long as these electrons can find partners. However, if there are too many unpaired oxygen atoms around, they can really be destructive to any bonds to which they are exposed. Oxidation can cause big molecules to break up.

In any chemical reaction that moves electrons from one atom or molecule to another, the one atom or molecule that gives away electrons is said to be oxidized. Rust, as an example, is the oxidized form of iron. The iron was oxidized because it lost electrons.

Reduction is the opposite of oxidation. What makes oxidization confusing (to me, at least) is that the atom or molecule that gains the electrons is reduced even though it gains an H+. (How can gaining be called reducing?) An atom or molecule in a reduced state has extra electrons to give away; one in an oxidized state has the ability to take or receive electrons.

Oxidative reactions generate energy when they release electrons; all metabolic reactions are oxidative, or energy-generating. Biosynthesis is a reductive activity: it requires absorbing electrons and takes energy.

NITROGEN FIXATION

What gardener doesn't know about rhizobia bacteria, the ones that fix unavailable nitrogen, making it available for use in plants? Guess what? They are endophytic bacteria! They live intracellularly in specialized root nodules, where they fix atmospheric nitrogen with little worry about competition or predation. This is not rhizophagy, but now that I know about it, nitrogen fixation seems pretty close.

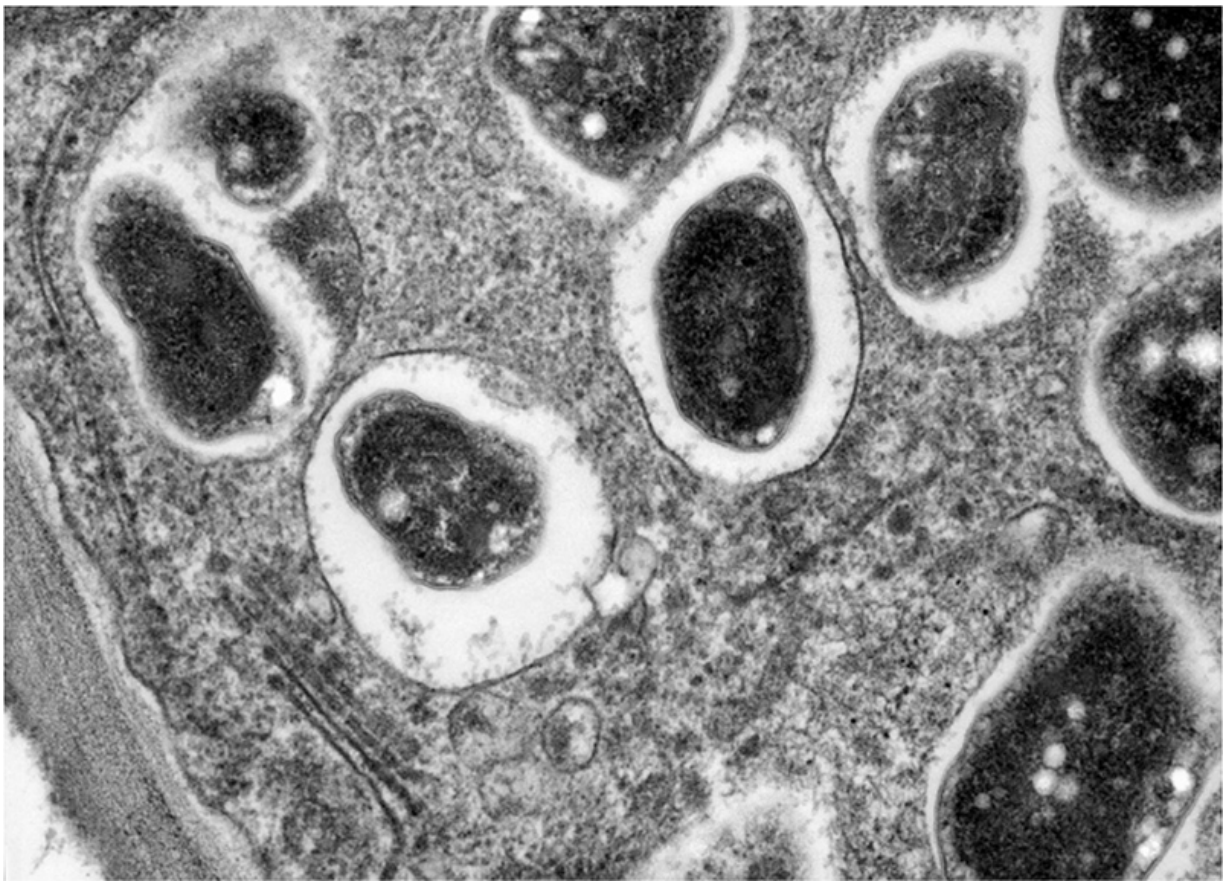
Nitrogen-fixing bacteria are a very important source of nitrogen, and not just for the bacteria. The plant host gets some of the nitrogen, and when the plant dies, its nitrogen ends up in the soil, where members of the soil food web put it into forms usable by a plant. Some rhizobia bacteria are free-living and fix nitrogen right in the soil; others need to form endophytic relationships with plants.

The symbiotic relation between a legume and rhizobia starts when a chemical produced by the legume (in reactions using lots of enzymes and amino acids) is perceived by the bacterium. The plant is in charge: it produces lipochitooligosaccharides (chains of sugars with fats, as you no doubt could tell) and structural molecules called nodulation factors (NOD). The plant has to let the bacteria know it is OK to enter it. The bacteria responds and communications ensue, until a root hair becomes a nodule. The point is, there is distinct two-way signaling between plant and bacteria. This is likely the case in rhizophagy too. How else would a bacterium be allowed into a plant, as happens in nodulated nitrogen fixation and rhizophagy?

In order to colonize a nodule, bacteria must enter the plant root hair. They do so via a tube created by the invagination of hair membrane cells. Once inside, bacteria morph into bacteroids, specialized symbiotic forms. Hmm. In rhizophagy, the bacteria are morphed into wall-less protoplasts.

Some non-leguminous plants (including maize, rice, sugarcane, and wheat) also host nitrogen fixers. In addition to rhizobia, *Azoarcus*, *Frankia*, *Gluconobacter*, *Herbaspirillum*, and *Klebsiella* are important endophytic nitrogen fixers in alder, bayberry, and even a few roses.

As to other sources of nitrogen: lightning creates a small amount of fixed nitrogen, and nitrogen can be fixed industrially, using the energy-intensive Haber-Bosch process. Biological production of fixed nitrogen is the most efficient source, and the process functions very smoothly: all fixation activities are regulated by turning genes on and off, expressing them, so they can produce the proper signals and synthesize the necessary molecules to pull the feat off. Obviously, a close dance has evolved between plants and bacteria.



Cross section of a soybean (*Glycine max* 'Essex') root nodule. This high-magnification image shows part of a cell with single bacteroids. The bacterium *Bradyrhizobium japonicum* infects the roots and establishes a nitrogen-fixing symbiosis.

Hooking up with a plant provides a bacterium a safe location and nutritional support; in exchange, the host plant gets nutrients from bacteria living inside the plant and much-needed nitrogen. There is even breeding and population-building inside the plant. All this sounds suspiciously like rhizophagy—which should make the fact of this new concept less surprising.

CHELATION AND SIDEROPHORES

Chelates are circular molecular compounds that consist of a metal atom attached to a ligand (aka chelant, chelating agent). These large molecules can be proteins, long chains of sugars (polysaccharides), and even nucleic acids. Many of the atoms in chelates donate electrons readily to metals (which attracts metals to them), though a few accept electrons instead. Chelates collect and hold metals, even toxic ones. Plants and bacteria produce and release chelates to tie up specific molecules in the soil. Or they may actually put chelates out into the environment to suck up a metal that is then taken up as a nutrient.

Root nodules formed by nitrogen-fixing rhizobia bacteria.

Bacterial chelation (the bonding of metals together) is accomplished by the production and release of siderophores, special water-soluble chemicals. Chelation is one reason rocks weather into soil: the metals in rocks are chelated out by decay microbes. This leaves the rock susceptible to further decay, both chemical and physical. Eventually, it becomes soil.

As long as we are on the subject of soil: fertilizers often contain zinc, copper, iron, and manganese in chelated form. Phosphate salts are required to break up these metallic micronutrients, but, in the United States at least, phosphorus is no longer allowed in many formulations (bye-bye, NPK fertilizers?). It is a good thing bacteria can do the job—in the future, they may be the only way to go.

SLIME, SLIME LAYERS, AND BIOFILMS

Bacteria produce various sticky substances. Though all three are an extracellular material (essentially glycoprotein), slime, slime layers, and biofilms are not the same thing.

Basic slime is glycoprotein loosely attached to the bacterial cell wall. It is not rigid; it can bend to the shape of the bacterium. Slime's primary functions are to protect the bacterium from drying out and from predation. It is also used by bacteria to attach to each other and surfaces. For some bacteria, this slime helps form an outer shell, or capsule. Capsules are hydrophilic: they like water and help keep a bacterium from drying out. These coverings may also make it more difficult for other organisms to grab hold of the bacterium and eat it.

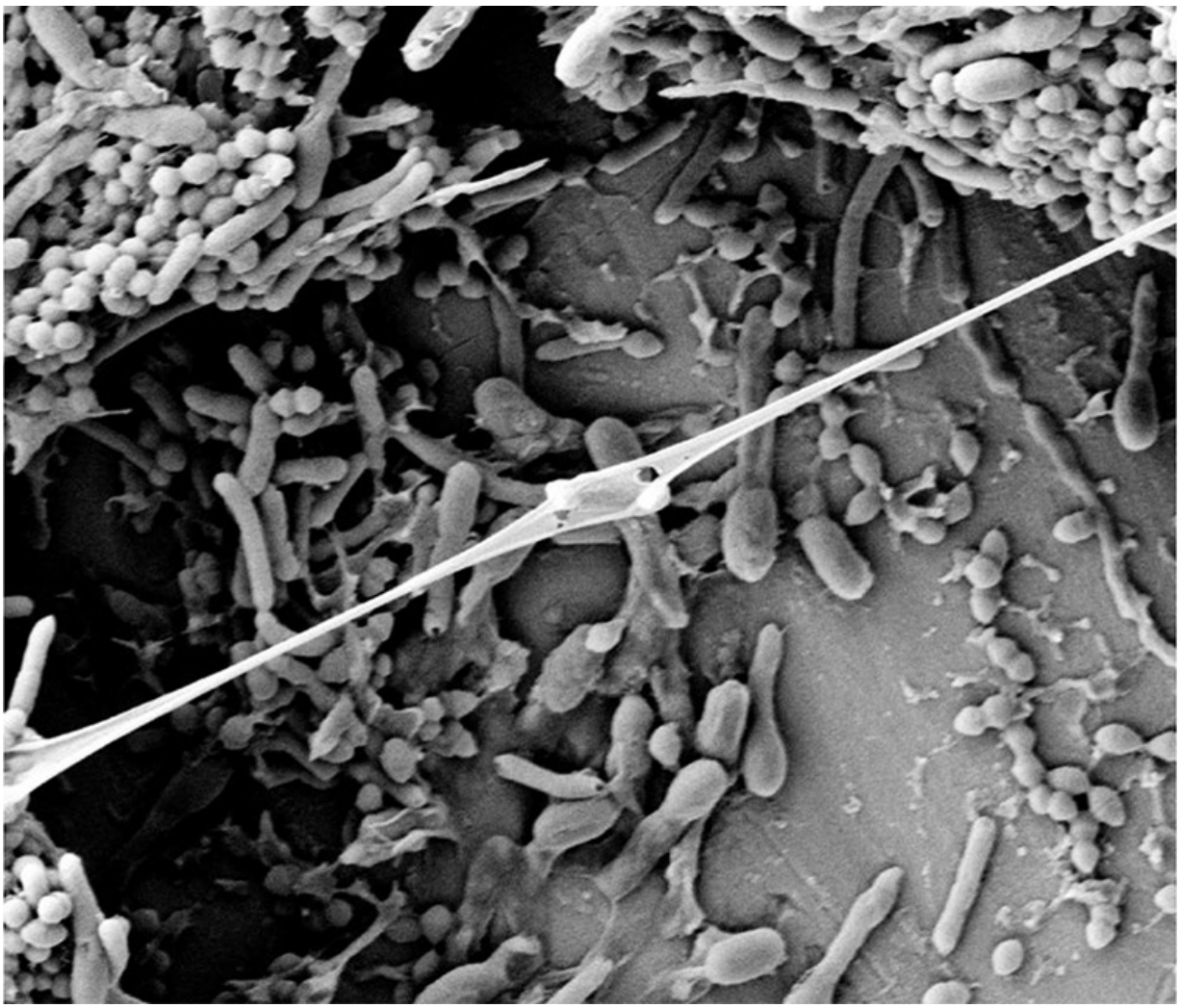
When you get enough bacteria (and enough slime), a slime layer forms. It generally consists of colonies of one kind of bacterium and can completely surround the cells but is relatively easy to get off when a bit of energy is applied. (Just scratch your teeth with a fingernail in the morning.)

As a slime layer thickens, it traps or attracts other kinds of bacteria. As populations diversify and grow, the glycoproteins produced build up. The layer becomes a biofilm (aka bioslime). This is much more difficult to remove than a slime layer—it's a job for a dental hygienist, not a toothbrush.

Imagine all manner of metabolic activity—waste generation, breeding, dying, policing, cleaning, feeding, transporting, and more—happening in such a confined area. A biofilm is a veritable bacterial metropolis thriving under a protective dome.

Under a slime layer and in biofilms, bacteria can live and thrive with some protection. They can also collect and accumulate nutrients. Formation of these layers is usually dependent on environmental conditions. All this is important because plants that use rhizophagy to obtain nutrients have

figured out how to use slime to work with bacteria to start the rhizophagy cycle (but let's not get ahead of ourselves).



Scanning electron micrograph of mixed-culture biofilm, demonstrating a spatially heterogeneous arrangement of bacterial cells.

ROS AND ANTIOXIDANTS

During the rhizophagy cycle the plant strips the cell walls off the intracellular bacteria. What is going on here? How does a plant cell strip the wall off of a bacterium, and how does it do so without also damaging its own?

Oxidation is the answer to the first question. Take away electrons from structural molecules in the bacteria's walls, and the walls oxidize and break

up. In response to the bacteria entering the cell, a plant makes a defensive move: it protects itself by producing reactive oxygen species (ROS).

ROS are oxidizing molecules, and oxidation reactions can be very destructive: they frequently release lots of energy, and their byproducts are often corrosive or toxic—even explosive, on a large enough scale. When bacteria enter the meristem root cell, it triggers the cell membrane to produce ROS and put them out into the periplasmic space (the area between the cell wall and the cell's membrane), where they break the bonds of the bacterial wall—which become toast (as it were) for the hungry meristem cell. The nutrients from the disassembled bacterial wall are carried out of the periplasm, across the plant cell membrane, and into the plant cell's cytoplasm, where they can be used for biosynthesis.

These ROS are not strong enough to destroy the plant cell's own wall, just the bacterium's. (The plant isn't stupid.) Better put, the plant has developed resistance to ROS, which are utilized by plants to deal with some stresses.

At the same time, some carbohydrates in the plant cell wall may be dissolved by ROS. These carbohydrates feed the intracellular bacteria, so they stay alive and can replicate. Perhaps these carbohydrates are fed to the trapped bacteria as an inducement to enter the cell and to keep them alive while they are there, without access to soil nutrients.

The antidotes to ROS are antioxidants, which neutralize ROS. The goal inside the plant is to reach a balance between ROS and antioxidants. This is homeostasis. Examples of antioxidants you might have heard of are vitamins A, D, and E, lycopene (found in tomatoes), beta-carotene (from carrots), and even individual elements, such as manganese and selenium. Enzymes (peroxidase, catalase) can also be considered antioxidants.

For the study of the rhizophagy cycle, it is enough to know (thank goodness) that the cell walls of bacteria are destroyed by a specialized ROS, superoxide (O_2^-). A plant stops an invading horde of pathogens by producing superoxide and using it in the periplasmic space.

NADPH OXIDASE ACTIVITY

NADPH stands for nicotinamide adenine dinucleotide phosphatase—a mouthful you can swiftly forget. What we need to know is that NADPH oxidase is a big, complex protein (an enzyme, actually, as you can tell by the -ase in phosphatase and oxidase). NADPH oxidase molecules sit in the

plant cell's plasma membrane and enzymatically generate superoxide, which specialized ROS is great at destroying the walls of intracellular bacteria.

NADPH oxidase is assembled in the cell's cytoplasm. Its activity has to be regulated to keep the amount of ROS in balance. Too much activity creates oxidative stress. Environmental factors as well as biological ones can cause NADPH oxidase—ROS production to get out of balance. Dealing with it during rhizophagy is like training a plant to deal with other stresses.

In plants (as opposed to animals, where this protein complex also exists), NADPH oxidase sets off a signaling trigger that plays an important role in cell growth. You will find NADPH oxidase activity during protein transcription and some intracellular signaling, not just when defense is needed.

ETHYLENE

Ethylene is a gas made up of hydrogen and carbon molecules (in other words, it is hydrocarbon). There are always four hydrogen atoms bound to two carbon atoms even though its chemical formula is H_2C (or occasionally CH_2). It is colorless and flammable. The precursor to ethylene is (do not panic, this is simply a ridiculous word I have to use) 1-aminocyclopropane-1-carboxylate (ICC), which comes into play later. It is all very complicated. You just need to know that plants produce ethylene. This ethylene is circulated around the cell and stimulates or triggers cell growth and other processes.

Scientists used to think that ethylene was produced only by plants. Its presence in soil that does not contain plants, however, was a pretty good tip-off that fungi or bacteria also produced it. Turns out they both do. If they can do so in the soil, they can do so endophytically, inside a plant cell, which they also both do.

Ethylene is a plant hormone, and hormones are signal molecules. In the case of ethylene, it is used to start cell growth, leaf and flower drop, and fruit ripening. Its presence influences growth of all plant parts. Exposure to ethylene can cause parts of plant cells to grow but also to die. (How contradictory.)

Using a particular exudate, the amino acid L-arginine, bacteria are able to synthesize ethylene in the soil. They continue to do so during rhizophagy, starting when they enter the plant. It is this ethylene that signals the plant

NADPH oxidase embedded in the plant cell's membrane to increase the production of superoxide.

Weirdly, the bacteria's own ethylene triggers the dissolving of its own cell walls. The nutrients in the bacterial wall are used to aid growth of the plant, but only after they move out of the periplasmic space, across the plant cell's membrane, and into the plant cell's cytoplasm.

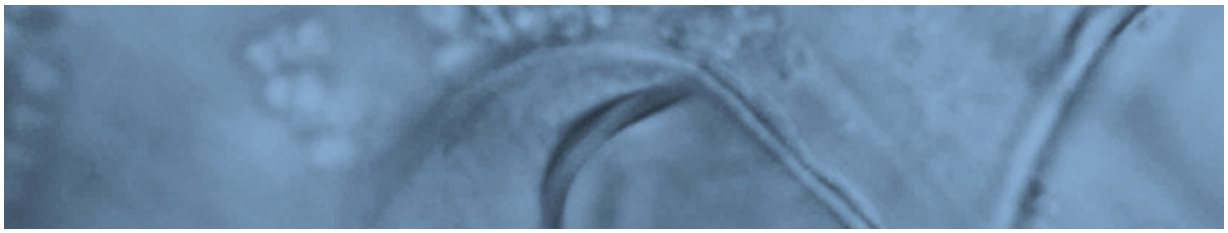
The wall-less bacteria are still alive and even multiply, but wall removal results in some leakage of metabolites. These too are transported across the membrane into the inner cell, where they too serve as plant nutrients.

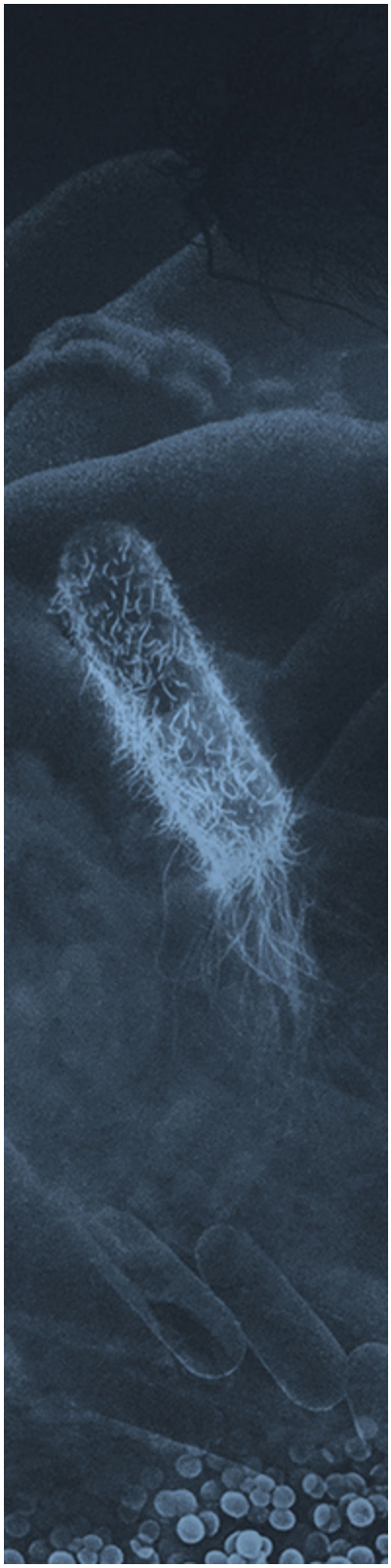
The answer to the question of why the plant's cellular walls are not also destroyed by ROS: the bacterial ethylene reacts with superoxide to form hydrogen peroxide (H_2O_2), a weakened form of superoxide. The ethylene acts as an antioxidant to the superoxide. The reaction that forms hydrogen peroxide produces carbon dioxide as a byproduct. This CO_2 combines with water to make carbonic acid. This is a weak acid and, by lowering the pH in the plant cell, the oxidation process slows even more.

Finally, we know ethylene also triggers biosynthesis of carbohydrates. These are needed for plant exudates. (Cake and cookies for soil microbes, as Elaine Ingham puts it.) Could the bacteria inside the plant cell be triggering the synthesis of exudates, specifically L-arginine, which they later consume when they are free-living in the soil so they can produce ethylene when they become intracellular again? It is circular but sure seems efficient.

ENOUGH!

You've been introduced to the chemical and biological concepts necessary to carry you through to a thorough understanding of endophytes and rhizophagy. I wouldn't use this to pass a high school biology or chemistry class, but I will review points as necessary (as promised)—and you can always dive deeper into any of these subjects to improve upon your understanding.





Bacterial Review

BACTERIA ARE SINGLE-CELLED prokaryotes, the fancy way of noting these organisms don't have a membrane-bound nucleus as do plant and animal cells (which makes them eukaryotes). Instead, bacterial DNA resides in the cell without a membrane protecting it.

It isn't easy getting a fossil of a bacterium, but the story is thought to have started some three to four billion years ago, with lifeless soap bubbles that morphed into membranes capable of trapping other forms with the right chemistry to create life. So, while bacteria have been around a really long time, they didn't come into focus (sorry) until the 1600s, when Antonie van Leeuwenhoek, a cloth merchant, discovered them while examining pond water with a homemade microscope. He was totally captivated by the movements of what we now know were bacteria. Imagine what he would think today, with all the advances in microscopy, the latest of which enable us to see the internal parts of these tiny organisms (and how they operate) in incredible detail.

Bacteria are fascinating creatures. They decay the organic matter in soil, disassociating matter into molecules which then become available for plant uptake. Bacterial slime builds soil structure by sticking soil particles together. And the humus we gardeners all seek appears to be an accumulation of dead bacteria that can't be decayed any further.

Finally, you may have heard that the body of every gardener has more bacterial cells than human ones. Some do the same things as endophytic bacteria in plants: they live in our bodies, increasing tolerances, neutralizing or destroying pathogens, digesting matter and putting it into usable form, and so much more. We humans are a lot like plants, thanks to bacteria.

THEY'RE EVERYWHERE

Why are we so ignorant of how bacteria operate? Is it because some can cause diseases and we don't like to think about them (save how to kill them to keep healthy)? It must be their size. They are just so darn small. Even under a 1000× power microscope, where plant cells look huge, bacteria are

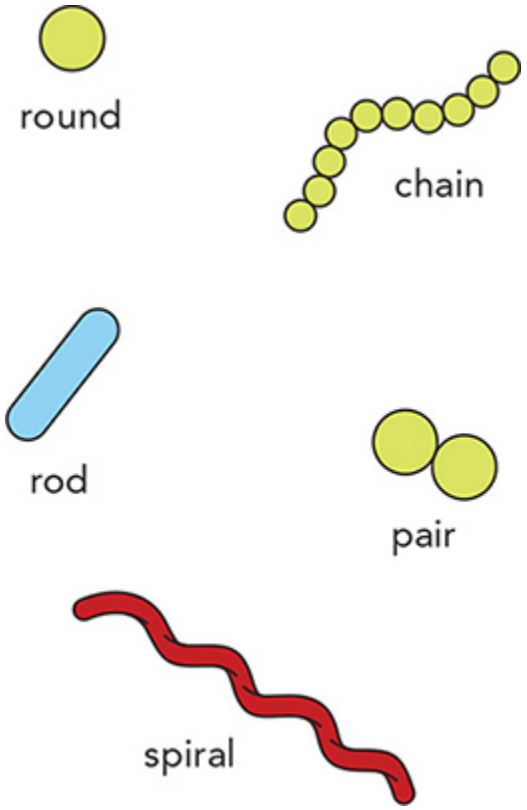
mere dots. In the process of writing this book, however, I have seen so many photos and videos of individual bacteria that they have gone from being little black dots to pet-size creatures—and they are amazing. If you cannot imagine what a bacterium looks like, you can't understand what it does. It is time to start envisioning and understanding them, gardeners.

Bacteria are measured in microns or µm (one-thousandth of a millimeter) or, because they are so small, nanometers, nm (one-millionth of a millimeter). On average they are 0.2 µm wide and 2.0 µm long, depending on shape. By way of comparison to a more familiar object, a single human hair is 17 to 180 µm thick—huge in comparison to a bacterium, but even this doesn't really create the proper mind's impression. To put it in perspective, if that human hair was the size of a towering pine, an endophytic bacterium would be the size of the bark beetle that might bring it down someday. Not good enough? Then again realize there are thousands of bacteria on the back of a flea and thousands on the tip of a pin. Try and keep these images in your mind: they will help you visualize how tiny a bacterium is and how many bacteria can be in just one small colony.

Small as they are, bacteria still come in various shapes, generally round spheres (cocci), rods (bacilli), and spirals (spirochetes). There are other shapes too—triangles, squares, filaments (filamentous)—and some bacteria even shape-shift.

No matter their shape, bacteria multiply quickly given the right conditions. On average, bacteria are capable of doubling their populations every 20 minutes or less. Do the math starting with one bacterium and see how large your population grows during a day. It is no wonder they are the most plentiful organisms by mass.

There are 500 million to a billion bacteria in a teaspoon of good soil (another reminder of their size). To get a better handle on what that really means, their weight per acre is equal to two cows (if you have good soil and decent cows). That's a lot of bacteria, considering their size. As for total numbers on our planet, it has been estimated that there are five million trillion trillion of them (5 followed by 30 zeroes)—an unfathomable number.



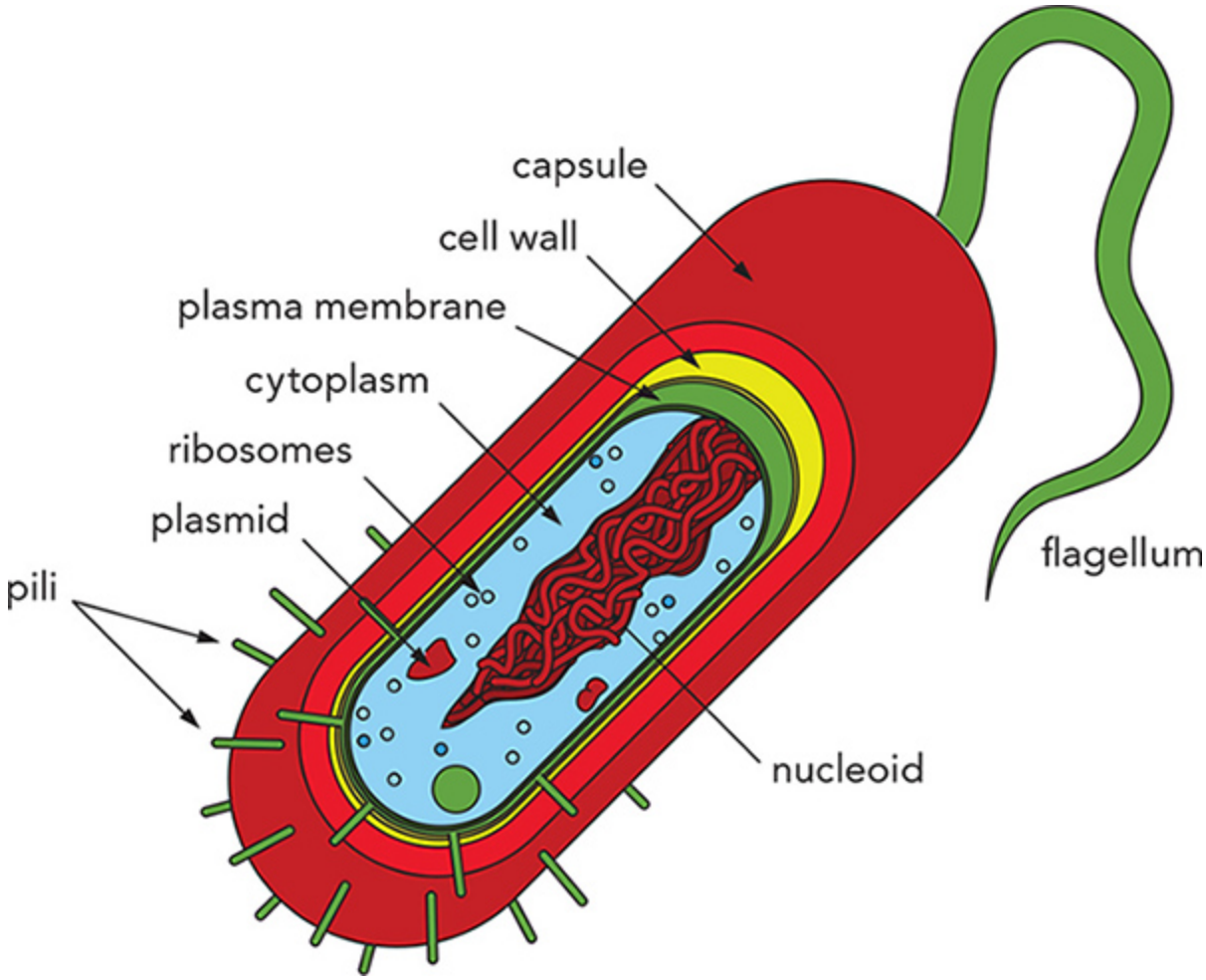
Bacteria: shapes and combinations.

SINGLE VS PLURAL

There is a difference between a single bacterium and a group of bacteria. It makes a difference if you are visualizing only one bacterium in a teaspoon of soil or the half a billion or so sitting in there. When you think about a bacterium, you should see a single cell. It is like one car in a driveway instead of a parking lot full of them. Of course, that bacterium you are visualizing won't be solitary very long. It will divide and divide again several times before you finish reading this book.

Bacteria (plural) denotes populations, one bacterium, and another, and another, in a group. Consider in your mind's eye how those half a billion bacteria in the proverbial teaspoon of soil move about in such a huge group (and what happens after 20 minutes).

Bacteria may be small, but they are distinctly three-dimensional. And they live in three-dimensional environments. You have no problem visualizing dinosaurs walking around even though they are flat on the pages of a book or up on the screen. Think about bacteria as 3D organisms, moving about in exactly the same way.



Parts of a bacterium.

A BACTERIUM'S WORLD

The environment a bacterium lives in is foreign to us. For one thing, the physics of everyday living are very different for something so small. For example, when a bacterium moves through its watery film in soil or in a plant, it has to deal with viscosity. To really understand this requires knowledge of fluid dynamics—not a physics lessons I am capable of getting into. But imagine this: bacteria literally pull fluids with them as they move. If you are swimming laps and stop moving, you will glide a body length or two before fully stopping or sinking. Contrast this with a bacterium that stops swimming. It continues to glide less than a millionth of its length (which happens to be the width of an atom). This is the impact of viscosity on small creatures. When you are dealing with viscosity, being svelte and

streamlined does not help with movement. Again, this is because bacteria are so small that drag or resistance is not a factor.

In sum: movement is tricky for small things. You know what it is like to wade through deep water. The smaller you are, the more difficult it is. And there you have it. Evolution had to develop ways for bacteria to move about and body features to assist.

FLAGELLUM

To be able to move in viscous environments, bacteria developed flagella (plural). Each flagellum (singular) consists of a long, hollow filament, a flexible hook at the end of the filament, and finally, a structure known as a basal body, which acts as the engine. Size of a flagellum? On average 0.02 μm wide and 10 μm long. Wow, really tiny!

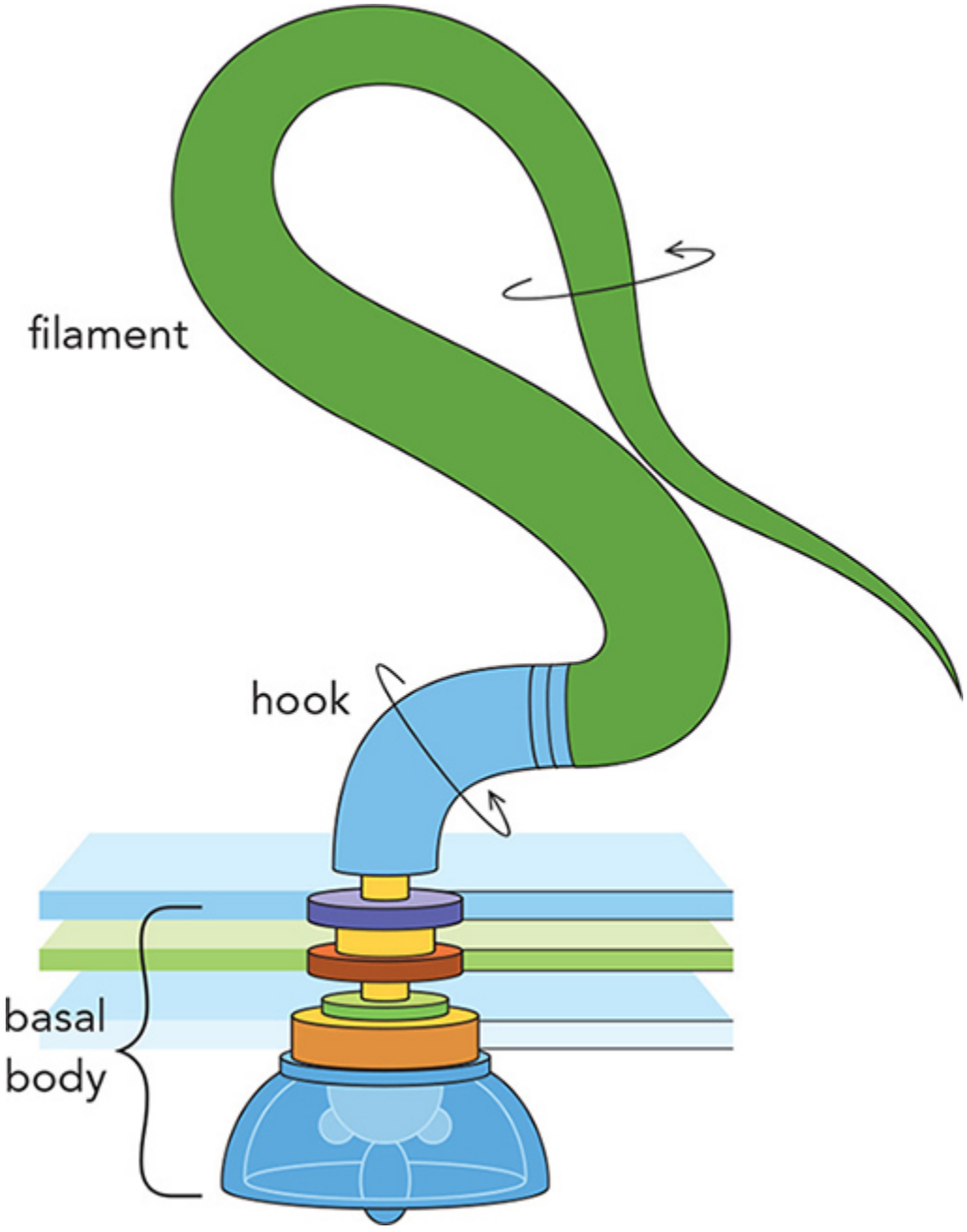
While some bacteria move by skating or gliding, and others use their distinctive shapes (spirals, for example, can move like a corkscrew—which it turns out is pretty efficient in a viscous liquid), most bacteria use at least one flagellum. Nothing demonstrates the sophistication of biosynthesis by bacteria (nor the power of evolution) better than these incredible structures. A flagellum is an amazing machine, operating as a propeller, not as an oar or whip.

Why do bacteria use propellers instead of whips or oars? In their viscous world, moving an oar back and forth would just make the bacterium rock back and forth in place, without forward or backward advancement. A propeller motion, however, provides a very efficient method of movement for bacteria. This has to do with the forces of drag on the length of the flagellum and its width.

Flagella can be found at either end of a bacterial cell and sometimes both ends. Some bacteria are covered with flagella. This makes sense as it is difficult if not impossible to reverse motion in a viscous fluid with just one flagellum.

A great deal of a bacterium’s protein synthesis goes into making flagella. One estimate is 7 to 9% of all the bacterium’s proteins are flagellin, the protein used to build flagella. There has been a great deal of study of flagella, not only because they are so sophisticated in their construction and mechanics but because they are so damn amazing. No other organism in nature uses a rotary motor for movement.

Every flagellum is a two-part rotary motor (filament and basal body), joined by a flexible hook. Rotation is provided by the basal body, a shaft surrounded by rings of proteins. Some bacteria have two rings and some have four, depending on if they are Gram-positive or Gram-negative. Operation of flagella involves really fascinating mechanics.

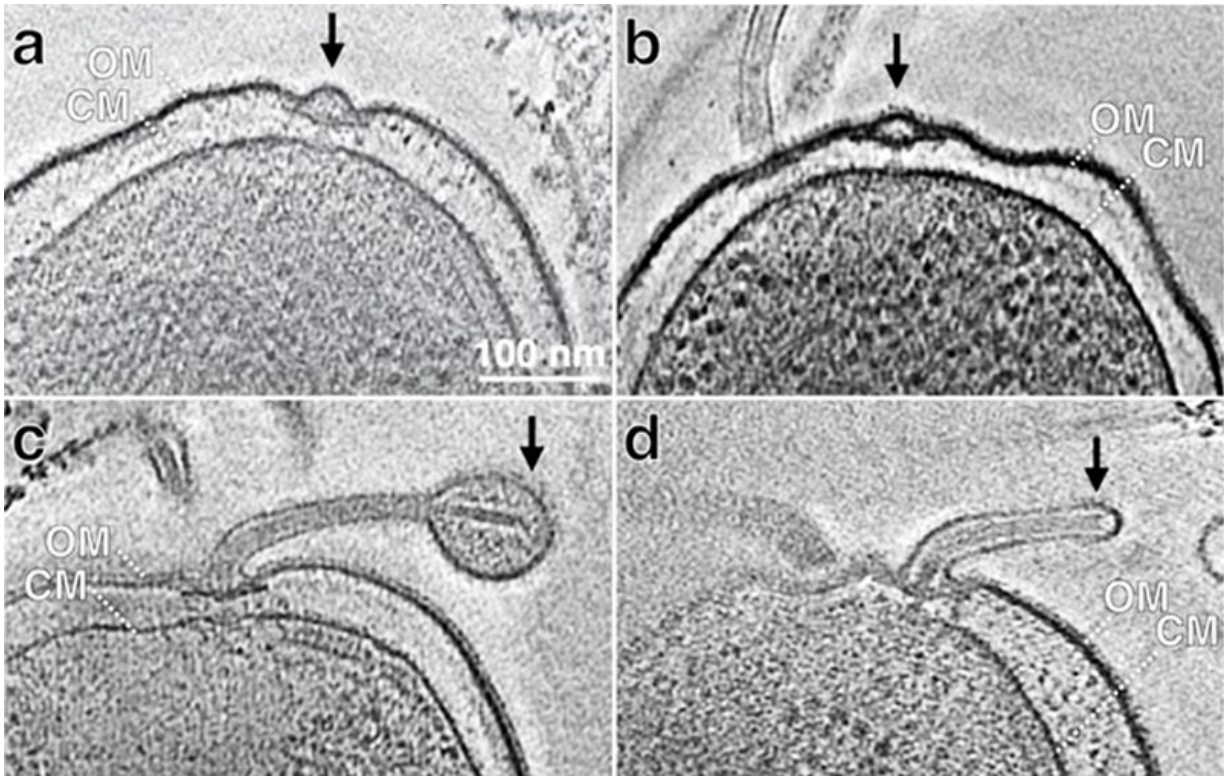


Detail: the amazing machine that is the flagellum.

The power to operate flagella comes from positively charged hydrogen protons that gather outside the cell membrane and interact with proteins in the membrane as they move inside. About 1,000 protons are needed to rotate a flagellum one turn. Wow.

The end result—proton motive force—rotates the filament (in either direction) up to 100 times per second at 1,000 rotations per minute, which is equivalent to 6,000 rpm for the motorheads out there. (Try running your car's engine at this speed.) As a result, a bacterium can travel 50 or 60 nm per second, a whopping hundred or so body lengths. A cheetah, the fastest animal, can only move 20 to 25 body lengths per second.

Moving at such fast speeds, you would think bacteria would collide all the time. Actually, they can sense materials in their environment thanks to chemoreceptors embedded in their cell membranes. Some of these embedded sensing proteins bind to external proteins, which causes the bacterium to change direction (think dragging a paddle while canoeing).



Flagellum formation.

PILI

Bacteria use the numerous thin hairs or filaments outside their cell walls to attach to each other as well as to random surfaces. These are pili. They are made of proteins and are fragile, so they need to be replaced often. Sometimes a mutation occurs in the new pili, and if it serves the bacterium well, it becomes yet another manifestation of evolution.

Pili are how a bacterium hooks up with another bacterium to transfer DNA. Pili thus involved in this conjugation are called sex pili. They attach to the second bacterium and bring it closer; once there is contact, a pore forms that allows the transfer of DNA. Different species can be involved, and this explains how strains are made. Conjugation (and a good dose of natural selection) is how bacteria develop new traits. In addition, pili possess binding sites at their tips, which help trigger immune responses.

Attachment pili (aka fimbriae) simply affix to a surface. They are usually short, and their proteins are stretchable. These pili allow the bacterium to pull itself toward the attached object (or vice versa). Nice to have a choice, I suppose.

THE CELL WALL

Bacteria build walls around their cells as do plants. These are mesh-like, porous, and one of the distinguishing characteristics of plant and bacterial cells versus animal cells, which lack outer cell walls.

The bacterial cell wall acts just as a plant's cell wall. It protects the contents of the cell from spilling out and determines the cell's shape. There is a lot of pressure inside a cell; in some bacteria, the osmotic pressure is comparable to air in a well-inflated tire. Cells would explode (osmotic lysis) in many environments if their contents were not contained by walls.

Around 90% of the bacterial cell wall is a chemical combination of eye-crossing words cross-linked with pentapeptide side chains (which are found in all protein molecules). Let's skip the breakdown this time; I'll just note that this particular nightmare is a combination found only in bacterial cell walls.

For our purposes, think of bacterial walls as very strong, lattice-like 3D structures of two basic types, which react differently to enzymes, to antibiotics—and to a certain purple dye. Indeed, bacteria are classified based upon the ability of their cell walls to take up this particular purple dye.

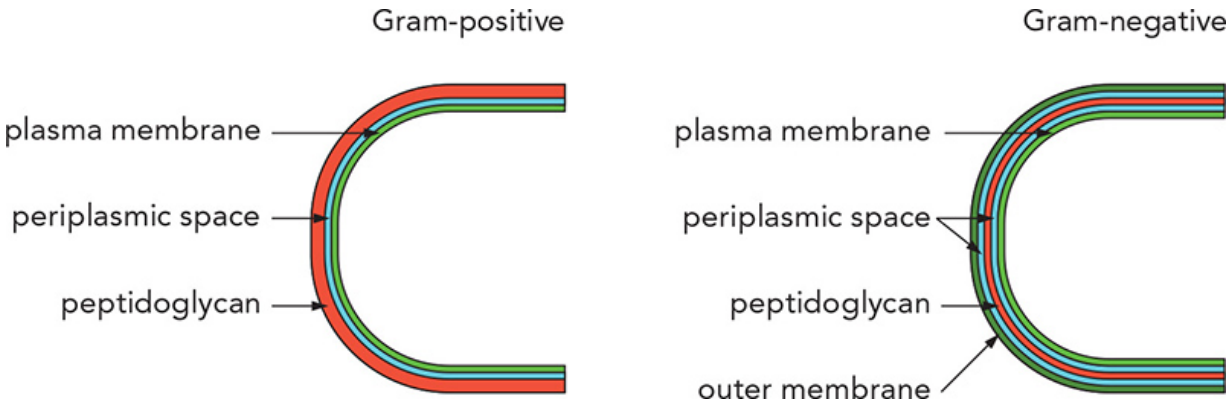
GRAM-NEGATIVE VS GRAM-POSITIVE

In 1884 Christian Gram discovered he could stain bacterial cell walls with a dye then being used as an antiseptic. (Anyone remember the sting of purple iodine?) He found the two colors that resulted from his staining corresponded to the two different types of bacterial cell walls.

The difference in ability to hold the dye is due to the different composition of the cell walls. Both contain amino acid chains mixed with carbohydrate chains (glycans). Gram-positive bacteria cell walls are thicker than Gram-negative and 50 to 90% peptidoglycan; they stain purple. Gram-negative bacteria (albeit they have an outer membrane) are thinner overall and only 5 to 10% peptidoglycan; they don't retain as much of the dye, so they stain pink.

Peptidoglycan is porous. Molecules 2 nm or smaller can pass through a typical peptidoglycan cell wall. Larger molecules need the help of exoenzymes; these proteins are produced inside the cell and secreted outside to break down large molecules.

While as much as 90% of their walls are peptidoglycan, Gram-positive bacteria have glycopolymers mixed in; these long chains of carbon-based molecules add rigidity to the wall and put a negative charge on the bacterium. Glycopolymers form teichoic acid structures, which protect the bacterium from concentrations of salt and high temperatures.



Gram-negative and -positive walls.

Gram-negative bacteria form a special outer membrane anchored to the wall by proteins. This outer membrane can trigger immune responses in infected hosts; it can also release toxins. And remember the rings at the base of flagella? Gram-positive bacteria need only two to seal off the machine that is the flagellum; Gram-negative need four.

One more thing about Gram-negative and -positive bacteria. Bacteria predators (and some of these can be cannibal bacteria) have an easier time with Gram-negative bacteria cell walls and can easily dissolve them. Predators of Gram-positive bacteria encounter thicker, tougher walls and require special proteins to soften them in advance of a direct attack.

Bacterial cell membranes are double-layered, as are animal and plant membranes. While animals and plant membranes have two layers of phospholipids, bacteria have only an inner phospholipid layer. The outer lipid layer is made with sugars, not phosphorus, so they are glycolipids, not phospholipids.

As with the cell membranes of animals and plants, both Gram-positive and Gram-negative cell membranes are embedded with various molecules that serve as porins (letting in moisture) and channels for nutrients, but they mainly serve as a protective barrier.

PERIPLASMIC SPACE AND PLASMA MEMBRANE

Gram-positive bacteria have only one membrane enclosing their cytoplasm. The area between this membrane and the cell wall is the periplasmic space (aka periplasm), an intracellular space where rhizophagy bacteria hang out. In Gram-negative bacteria, however, there is also an outer membrane, on the outside of the wall. The periplasmic space is still between the wall and the plasma membrane, however; in either case, it is filled with a viscous, aqueous solution that is loaded with proteins.

Inside the cell wall of a bacterium, on the other side of the periplasmic space, is the bacteria’s plasma membrane. This consists of two sheets of charged, lipid molecules with spherical heads and rod-shaped tails. This membrane operates in the same way as animal and plant cell membranes; however, while animal and plant membranes are phospholipids, bacterial membranes can contain any number of different fatty acids. These can be adjusted by the bacterium to ensure the membrane is fluid.

The membrane regulates what gets in and out of the cell. There are specialized, embedded proteins that act as channels that allow material to move in and out passively or more complicated pumps that require energy to operate. Plasma membranes also contain embedded structures that serve as signaling or communication systems between the inside of the cell’s cytoplasm and the outer world.

Remember that Gram-negative bacteria have two membranes, plasma and outer. The porins or channels are in the outer membrane. The sugars and proteins that enter often stay in the periplasmic space between the two membranes. The periplasm's gel-like fluid is filled with many proteins. It also contains peptidoglycan for wall-building and maintenance, and the specialized proteins that go through the layers serve as signal transporters.

CYTOPLASM

The cytoplasm is the liquid portion of the inner bacterial cell contained by the membrane. The membrane is selective in what it allows to cross into the cytoplasm. While it is the source of most of the proteins and other molecules in the jelly that fills the periplasmic space, it is distinct. Bacterial cytoplasm is mostly water, about 80%. Floating (not really the right term, but you get the idea) in it are enzymes and other proteins, nutrients, and wastes, as well as inclusions and chromosomes that carry the bacterium's DNA. All this is organized spatially, not just randomly.

INCLUSIONS

It is hard to give each of the tiny molecules found throughout bacterial cytoplasm a name; they can range from small assemblies to more complex structures that are almost like eukaryotic organelles. Some people call them compartments, but they are more often referred to as inclusions.

Inclusions, whether organic or inorganic, are found in the periplasm as well as in cytoplasm. An item has to be really small to be an inclusion, but size isn't everything when it comes to bacteria. For example, some inclusions are granules of a molecule used to make that odoriferous butyric acid. These granules are 0.2 to 0.7 μm across; they serve as long-term carbon energy storage.

Carboxysomes are inclusions found in all cyanobacteria and many bacteria that fix carbon dioxide. They increase the amount of rubisco (the main enzyme involved in carbon fixation) in the bacterial cell, allowing for more rapid CO_2 fixation.

Magnetosomes are yet another sort of inclusion, usually made of magnetite ($Fe3S_4$) molecules. As their name suggests, magnetosomes have something to do with iron; being magnetic, they orient the bacterium.

Finally, there are sulfur globules and polyphosphate granules; both sorts of inclusions serve as storage for inorganic materials used during biosynthesis. Bacteria that grow in sulfur-rich environments have globules of sulfur resulting from the oxidation of H_2S into sulfur; these sulfur globules usually reside in the periplasm. Polyphosphate granules are the source of the phosphate needed for biosynthesis of nucleic acids.

WALL-LESS BACTERIA

Ten percent of bacteria do not have cell walls. Some simply never had them, and others have had their walls removed for various reasons. A bacterium without a wall becomes spherical. As you can imagine, removing the cell wall weakens the organism. Bacteria can no longer respond to differences in ionic concentrations inside and outside the cell and become very sensitive to osmotic stress. Wall-less, the cell can still take in nutrients, but it leaks cytoplasmic fluids, electrolytes, and metabolites.

Some wall-less bacteria (aka spheroplasts, protoplasts) continue to reproduce, and they do it more quickly without having a cell wall to deal with. These L-form bacteria (after London's Lister Institute, so don't go looking for any deeper scientific meaning) are capable of dividing. L-form bacteria are not rare, but they are usually extremely difficult to see. They are necessarily smaller than walled bacteria, some extremely small at 0.01 μm in diameter, and they reproduce via budding or blebbing—which is what a yeast does: pinching off a part of itself. In blebbing, a small bud forms on one side of the mother cell; the bud grows until it is separated or made part of a chain.

While blebbing, L-forms can merge (hybridize) with other protoplasts. They can take up nucleic acids, proteins, viruses, chromosomes, and even chloroplasts by endocytosis (surrounding and engulfing). They can produce metabolites. They pretty much do what walled bacteria do.

ENDOSPORES

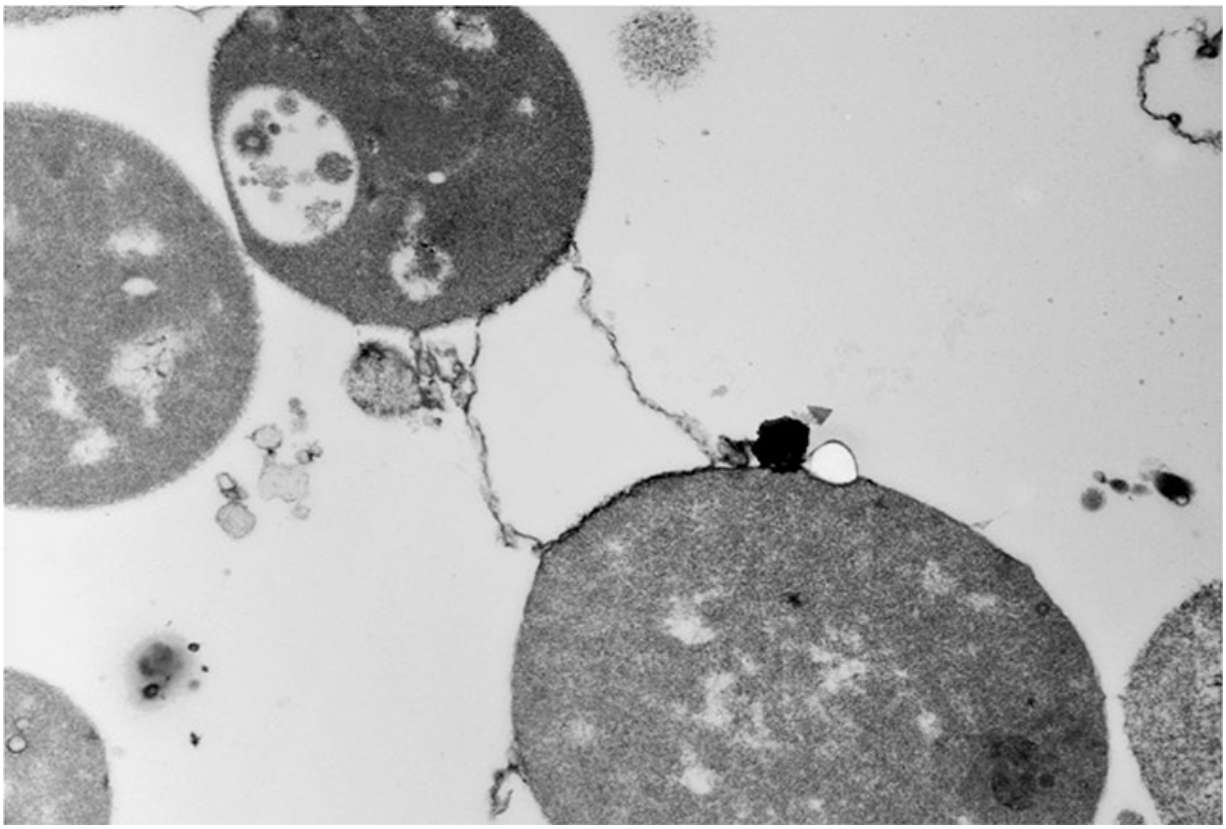
As long as we are dealing with otherworldly wall-less bacteria, let's include endospores, which are often classified as inclusions. Only Gram-negative bacteria form endospores, and not very many of these can make them. Some transform themselves into endospores when things are not going well. It is sort of like a caterpillar going through metamorphosis, only instead of a

genetic trigger, there is a stress—one caused by changes in pH, temperature, light, or nutrient availability.

Endospores are created inside a bacterium's cell; when finished they are inactive, and they remain in that state until they are moved outside the cell and dormancy is broken. Endospores resist antibiotics, heat, pH—the whole gamut of things that would typically kill a bacterium. *Bacillus* spp. in particular are capable of forming lots of endospores. It is a skill worth having when you encounter a neighbor that wants to kill you or an environment that, for one reason or another, is just too harsh.

RIBOSOMES

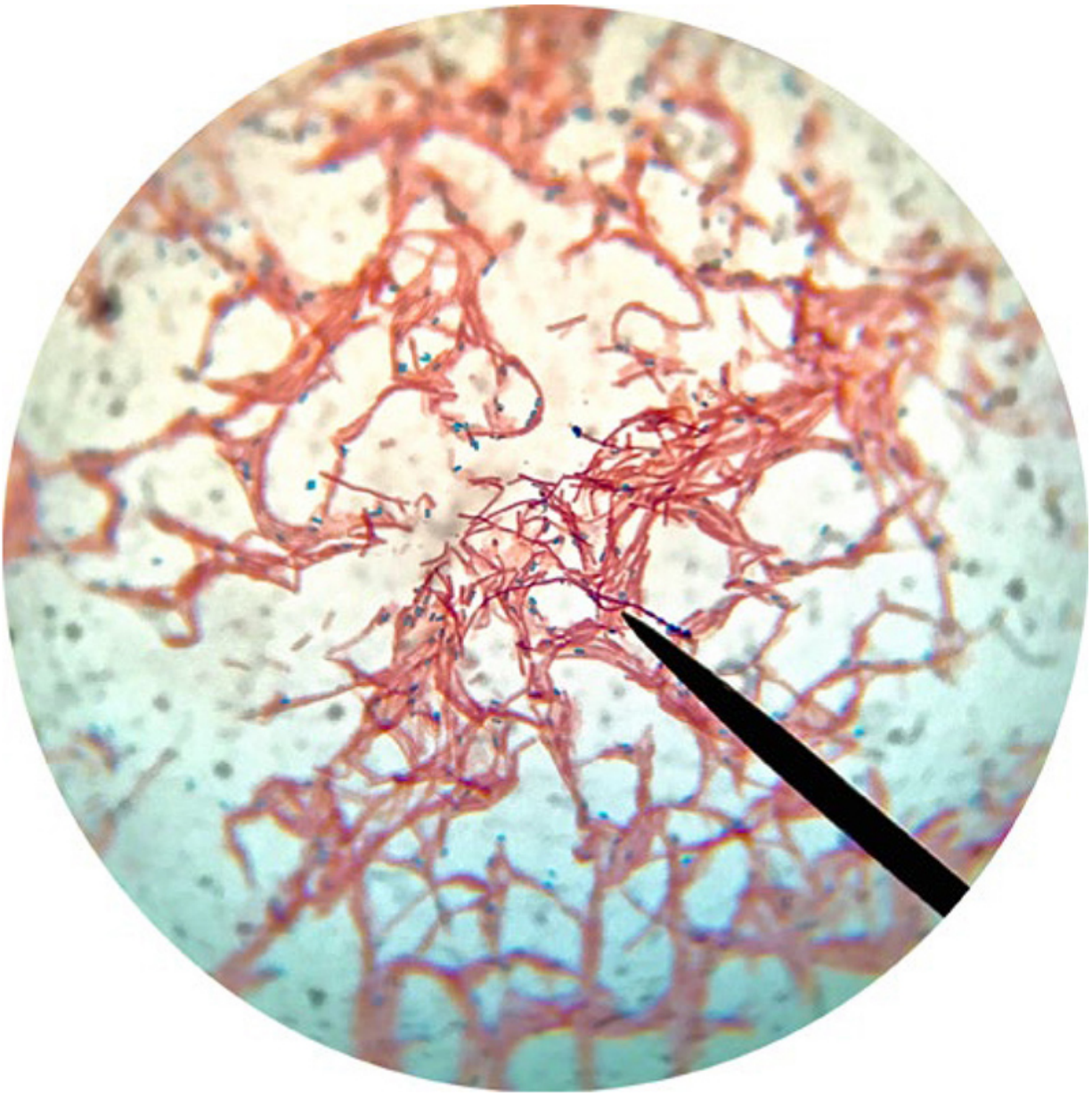
Like all living cells, bacteria have ribosomes, which assemble amino acids in accordance with blueprints found on messenger RNA (mRNA). There are more ribosomes in a bacterial cell than any other inclusions, usually a few hundred of them. Ribosomes assemble amino acids during biosynthesis. The end results are polypeptide chains, which are released into the cytoplasm. Consider that the ribosomes themselves are synthesized inside the bacteria cell, also from proteins. This requires somewhere in the vicinity of 200 different proteins and lots of energy. In fact, a staggering 60% of the energy used in the bacterium's cell is used to produce its ribosomes. They are extremely important, obviously.



L-forms are wall-less but functioning bacteria.

Bacterial ribosomes are 10 to 20 nm in diameter. That is small but, during biosynthesis, up to 20,000 can be active. It is thought that the reason bacteria don’t get larger is because there wouldn’t be enough room for all the extra ribosomes that would be needed to make a larger bacterium.

Bacterial ribosomes often clump together, forming polyribosomes or polysomes. These are comparable to a clump of beads. Each consists of two subunits, which must be combined for the ribosome to operate. Magnesium is involved in connecting the subunits, which appear during cell elongation.



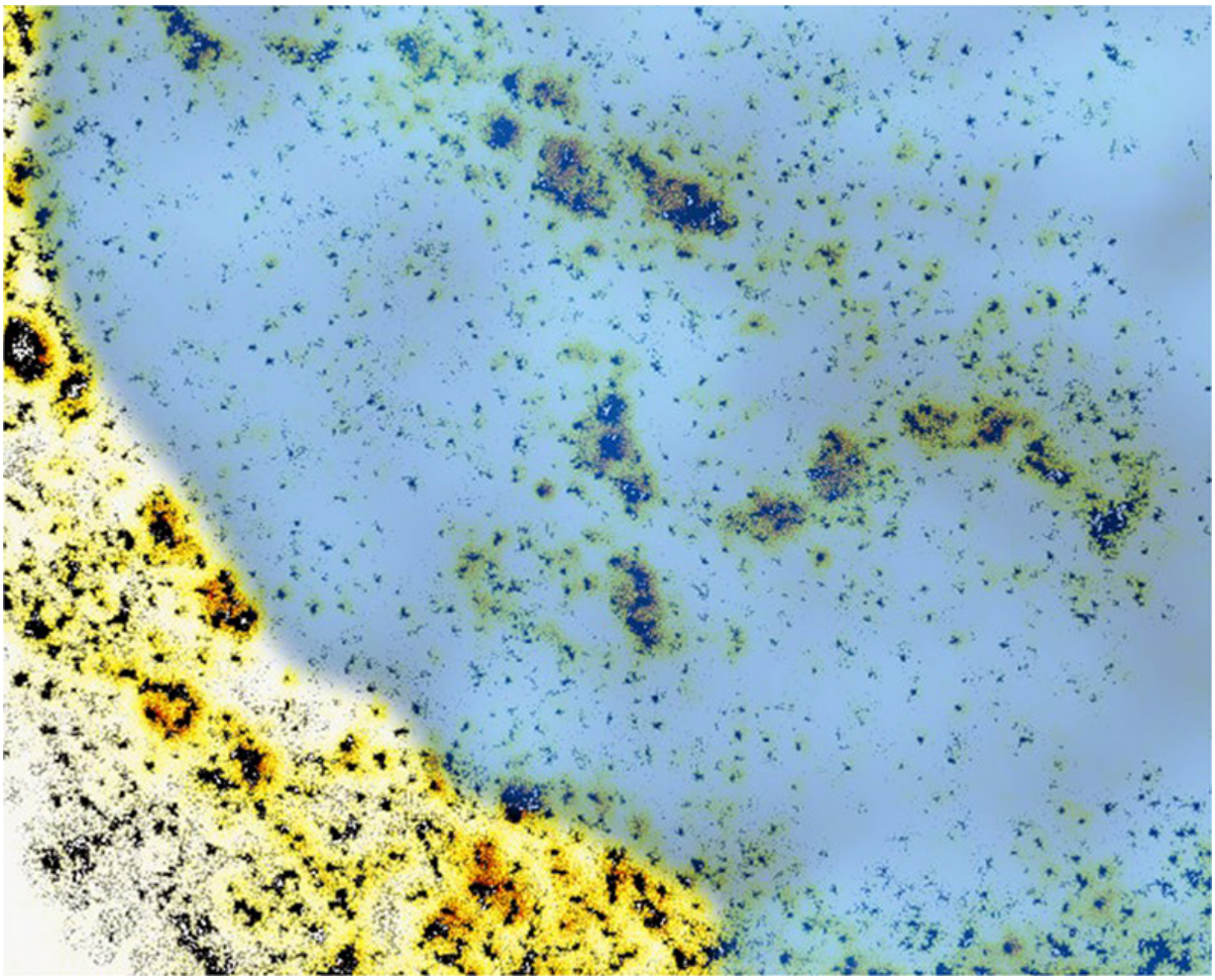
An endospore stain of *Bacillus subtilis*. Spores are green (malachite green stain); the *B. subtilis* cells, counterstained with safranin, are reddish.

FTSZ, MREB, AND CRESCENTIN

When I was in school, I was taught that bacteria lacked cytoskeletons, the infrastructure of fibers and filaments found in eukaryotic cells which protects them from external forces and helps them maintain shapes. It turns out with better microscopy we can see all manner of similar filaments in bacteria after all, cytoskeletons of sorts. These filaments—long chains of proteins extending throughout bacterial cytoplasm—are used during cell

division, help maintain cell shape, and protect the cell from being destroyed by external forces.

One such set of filaments is FtsZ (filamenting temperature-sensitive mutant Z), a protein encoded with a gene that is absolutely essential to bacterial division. FtsZ collects and organizes other proteins necessary for division and creates the septum that forms between dividing cells. FtsZ filaments are similar to actin in plant cells.



STED image of bacterial ribosomes.

MreB, another protein that is similar to actin, is coded to help provide shape and structure to non-spherical bacteria. It controls the width of rod-shaped bacteria, for example. It accomplishes this by regulating the enzymes that make the peptidoglycan used in the construction of bacterial cell walls. MreB is helical, and it runs up the body of the bacterium. This exerts outward pressure that prevents the cell from assuming a spherical

shape. Both FtsZ and MreB are key to the positioning of bacteria too. They control polarity that expresses front and back and top and bottom.

Crescentin, a third component of bacterial "cytoskeletons," grows lengthwise in a bacterium, causing the inner curve (as in a C) of crescent-shaped bacteria.

CHROMOSOMES AND PLASMIDS

Bacteria pass on and acquire traits via genes held in DNA's double-helix structure. The bulk of bacterial DNA sits in one large circular chromosome. The bacterial chromosome, along with proteins and RNA molecules, are situated in an area known as the nucleoid. There is no nucleus.

There are also small bits of chromosomal DNA arranged in circular shapes elsewhere in the cytoplasm. These are plasmids, and there can be hundreds of them in a bacterial cell. Plasmids don't carry the entire bacterial DNA code, but they can replicate independently. While there are many plasmids, there is only one bacterial chromosome.

An individual plasmid contains genes for the expression of only one to three traits, and bacteria could get along without them. However, plasmids have evolved to help in stressful situations, such as when the bacterium needs to produce a necessary antibiotic or needs an enzyme to digest a particular predator. Without plasmids' help, the life of the bacterium would be very different and more difficult, so bacteria have evolved to expend a lot of energy to support them.

One fascinating set of plasmids has the ability to poison their hosts if not supported. These feed the bacterium an antidote to a poison they produce. If the plasmids are not duplicated, there is not enough antidote. (Yikes!)

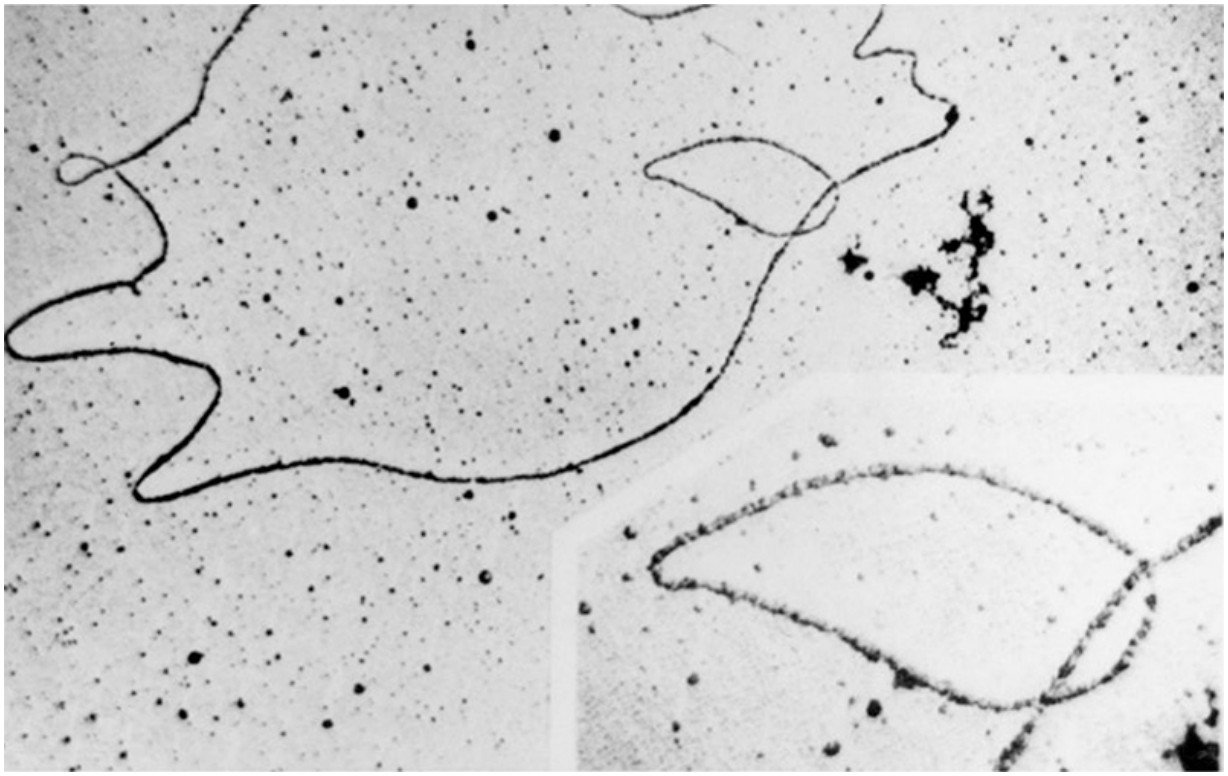
Plasmids duplicate inside the bacterium independently of the full DNA-containing bacterial chromosome. And, they can be passed to other bacteria asexually. Also, when bacteria divide in half via binary fission, plasmids can leak out. These are often picked up by other bacteria. (Here is where you should be visualizing a gazillion bacteria eating anything that is nearby.)

CAPSULE

Some bacterium produce a gummy layer of polysaccharides just beyond the cell wall that completely covers the cell. This is the bacterium's capsule

(glycocalyx to scientists, who always make it a bit more complicated). It is very tightly packed, so much so that it doesn't Gram-stain easily. This protective, hydrophobic coating is resistant to things like detergents, which exist in nature. (You didn't think Procter & Gamble invented them, did you?) The capsule sticks to the cell wall, keeping viruses out and moisture in. Pathogenic bacteria use their capsules to protect against antibiotics.

A capsule is difficult to get off a bacterium—it is that integrated with the wall. And it is sticky on the outside; this helps with its protective abilities, surprisingly, making it hard for an enemy to grip the bacterium to consume it.



Bacterial DNA.

S-LAYERS

S-layers (S for surface) are thin masses of proteins that are well organized but not so well organized as to form a crystal. They attach to the outside of the cell wall in some Gram-positive bacteria (and lots of archaeans, where S-layers can be the whole cell wall) and outside the outer membrane in some Gram-negative ones. An S-layer helps provide rigidity, protects

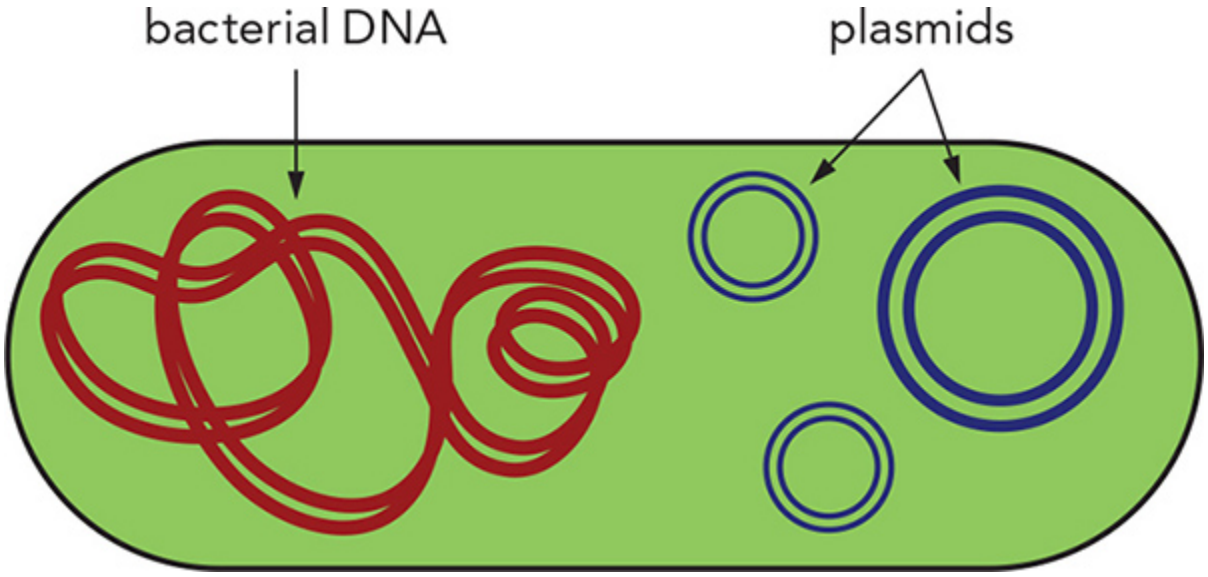
against some enzymes, and makes it more difficult for enemies to consume the bacterium. They also provide some adhesive properties.

QUORUM SENSING

How do bacteria talk to each other? No need to get overly complicated. Bacteria communicate via chemical signaling. In those groups of gazillions, they use these signals to engage in quorum sensing. Simply put, quorum sensing allows the group to act as one, like a flock of birds suddenly changing direction. The kinds of things that are quorum-sensed are processes that would not work with just one or two bacteria, things such as biofilm formation and bioluminescence.

Quorum sensing requires autoinducers, extracellular molecules that diffuse through cell walls—easily, in the case of Gram-negative bacteria. It takes more energy for the autoinducers to be moved across the membrane of Gram-positive bacteria, which have a high percentage of peptidoglycan in their thick cell walls.

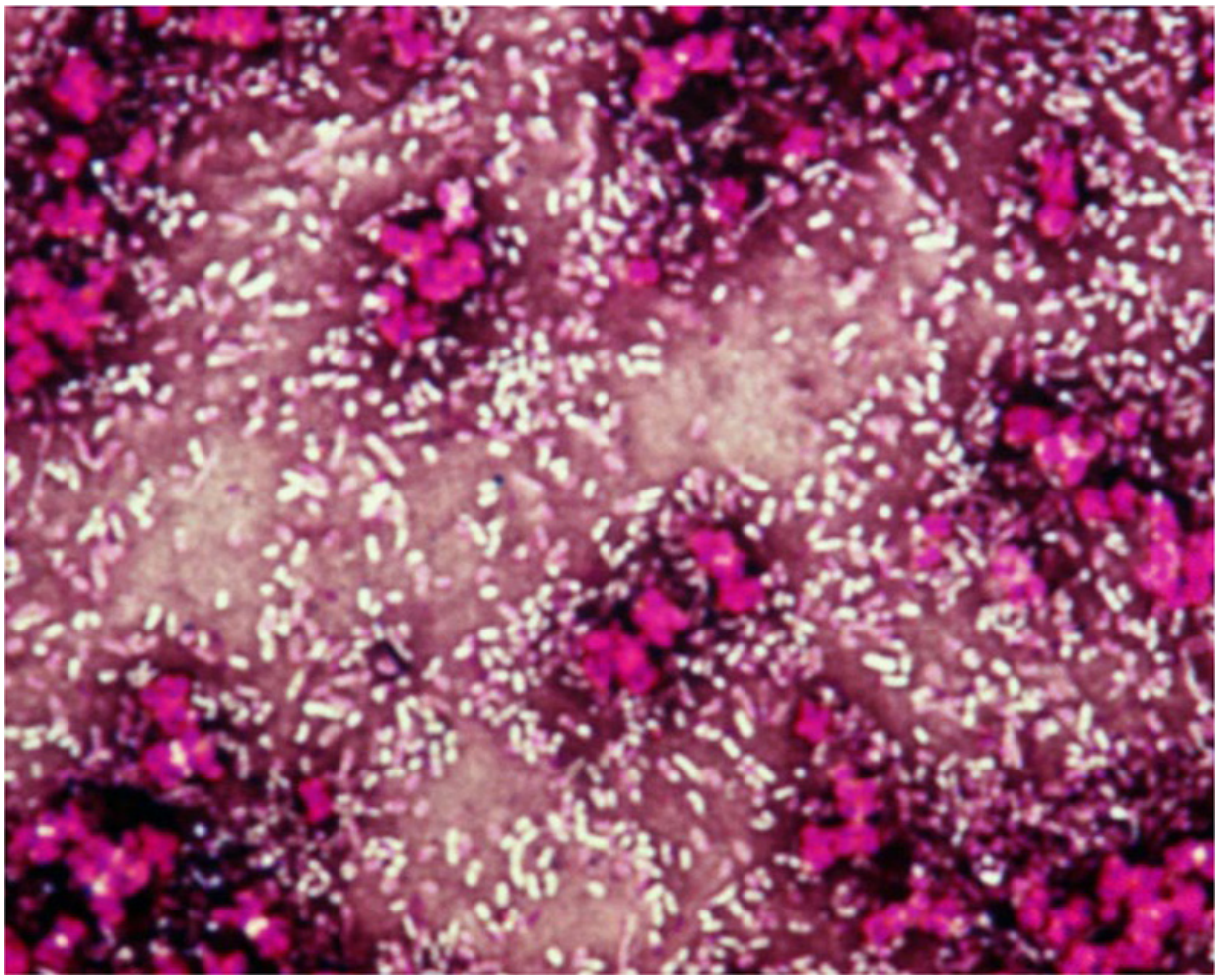
Different bacteria have different autoinducers because they need to sense different things. (Duh!) As bacteria divide, more autoinducers are put out into their environment. At some point there are enough that they stop moving out of the bacteria and start to accumulate inside them. This buildup causes changes in gene expressions and results in the bacterial reactions. Bacteria might become virulent and produce a toxin, or might produce a special enzyme to break down a compound. You get the idea (right?).



Each bacterial cell has a single loopy bacterial chromosome and several plasmids.

Some bacteria don’t have their own autoinducers; instead, they have special receptors to—you guessed it—sense and respond to the autoinducers of others. Smart.

Finally, quorum sensing communication can be between the same species of bacteria or interspecies. It can lead to competition or cooperation. This is a complicated area of science, obviously distilled way down here, but the takeaway is simple: bacteria communicate and do so far better and infinitely faster than we do.



A bacterial capsule at 4000 × magnification.

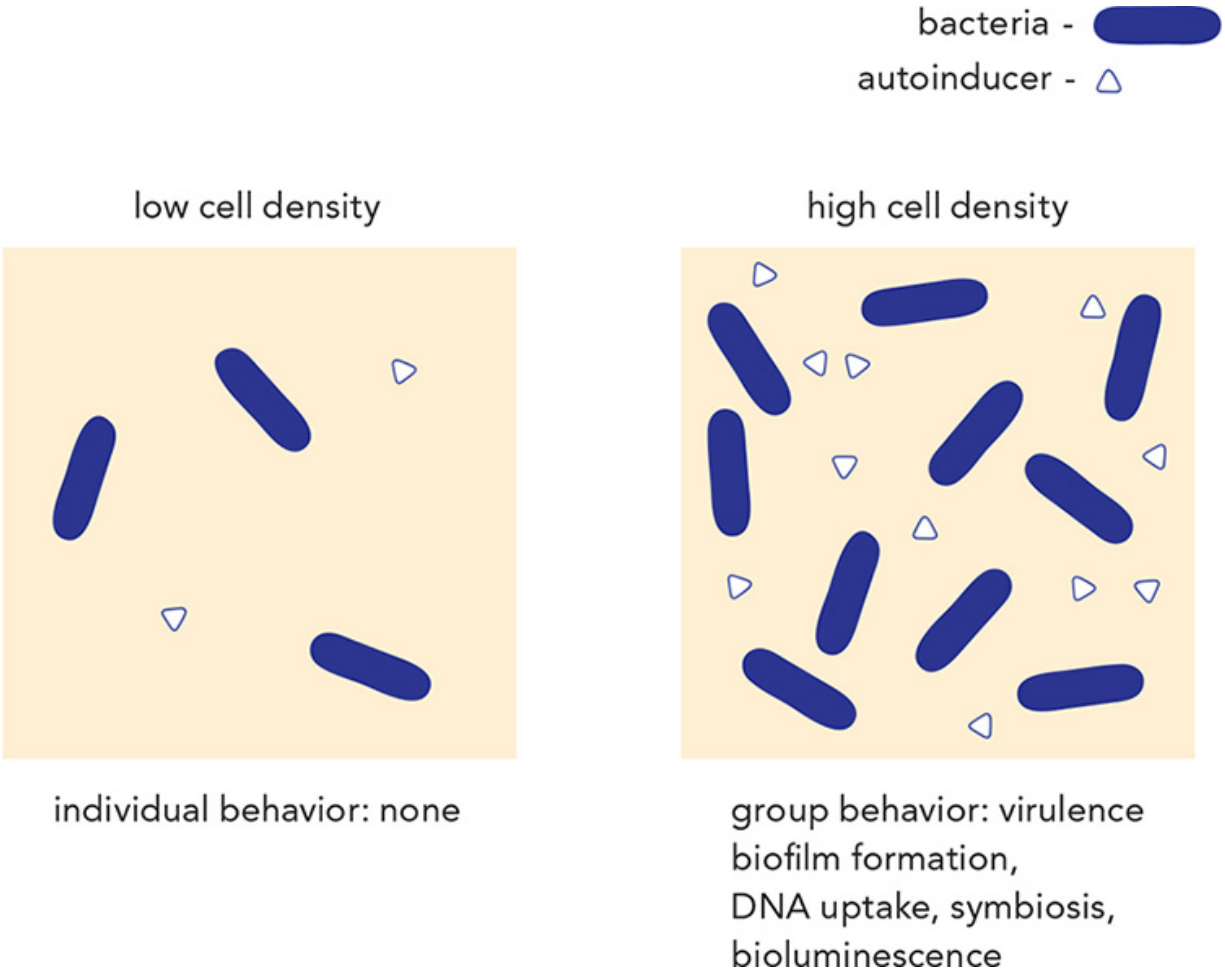
NUTRIENT ACQUISITION

All microbes need energy and nutrients in order to build the molecules of life and reproduce. Bacteria obtain these necessaries in three ways. Photosynthesizers harness the energy of the sun. Chemotrophs break down inorganic chemical compounds. And decomposers disassemble organic matter.

The primary element bacteria need is carbon. This is because the carbon molecule is the base of the four molecules of life (carbohydrates, lipids, proteins, and nucleic acids). Carbon bonds store energy. This is true of plants and animals as well.

Obviously, bacteria need other elements to create the molecules of life. Hydrogen and oxygen, for example, are used in most compounds. Phosphorus is needed for membranes. You can’t build amino acids without

nitrogen (and in some cases sulfur). Think essential plant nutrients, macro- and micro-.



Bacteria communicate via quorum sensing.

How do all nutrients, essential or otherwise, get through the cell membrane and end up inside bacteria? There are several ways, starting with diffusion (aka passive diffusion). Very simple, small molecules—particularly water and carbon dioxide—use this route.

Diffusion (and osmosis, which is diffusion for solvents) works because Nature seeks equilibrium. When the concentration of molecules is higher outside a cell than it is inside the cell, a concentration or energy gradient is created, and molecules from the concentrated side of a membrane move to the less concentrated side to create balance. This even works with a tiny bacterium.

When the higher concentration of molecules is inside the cell, nutrients pass across the cellular membrane with the help of carrier proteins located outside the cell. These enzymes move down their gradient (each substance has its own) but also carry nutrients that can't. This assist is facilitated diffusion, one of many types of passive transport.

And then there is active transport. This is like facilitated diffusion in that it works despite the reverse energy gradient. It does so because the bacterium expends energy to get the carrier proteins to beat the concentration gradient.

Iron, however, requires a special system. That is because iron in soil is ferric iron (Fe3+) (think rust). Siderophores are manufactured by bacteria and put into the environment where they chelate or bind to ferric iron. Then the iron is moved into the cell via a specialized receptor system. Sometimes it is reduced, solubilized outside the cell wall and then moved across the wall and the cell's plasma membrane. Or the entire mass of molecules goes through the cell wall into periplasmic space, where it is disassembled before its parts move across the plasma membrane.

REPRODUCTION

Bacteria double their populations every 20 minutes on average, even faster if they are wall-less. When they have walls, bacteria reproduce asexually, no partner needed. However, there is more than one way for a bacterium to transfer its genetic material.

Conjugation is the process of transferring genetic material via plasmids. It is a straightforward process. A pili secures itself to a neighboring bacterium. The circular, double strand of DNA in a donor's plasmid is cut, unzipped, and one of the strands moved to the new cell. The strand in the old cell duplicates itself, reforming in the normal double strand. In the new cell, the single strand is also duplicated and an identical plasmid is formed. Where there was one plasmid, there are now two. Some of this transferred DNA becomes incorporated in the bacterial chromosome.

Most bacteria can reproduce by engaging in a second asexual process, binary fission. This is like the division in our cells, mitosis. One cell divides into two. The daughter cells are the same size and volume and contain the same material.

That this replication routinely happens in under a half-hour from start to finish is amazing when you consider all that is involved. A tremendous

number of enzymes are employed, and all these have to be biosynthesized by the bacterium. Who said bacteria are simple organisms? Just knowing they have to replicate those intricate flagella blows my mind.

Iron bacteria in surface water.

METHODS OF CLASSIFICATION

Bacteria can be organized in lots of ways. For example, you can group them into functional categories based on what they do, such as decomposing. Chemoautotrophs are bacteria that use things other than carbon for their energy source. Mutualists, including many nitrogen-fixing rhizobia, form partnerships. Another group might be reserved for pathogens that attack plants.

It is also possible to classify bacteria based on their need for oxygen. Aerobic bacteria need oxygen to live. Anaerobic bacteria don’t; in fact, they

die in the presence of oxygen. A third group, facultative anaerobes, can function in the absence of oxygen but almost always do better if it is present.

BACTERIA AND THE SOIL FOOD WEB

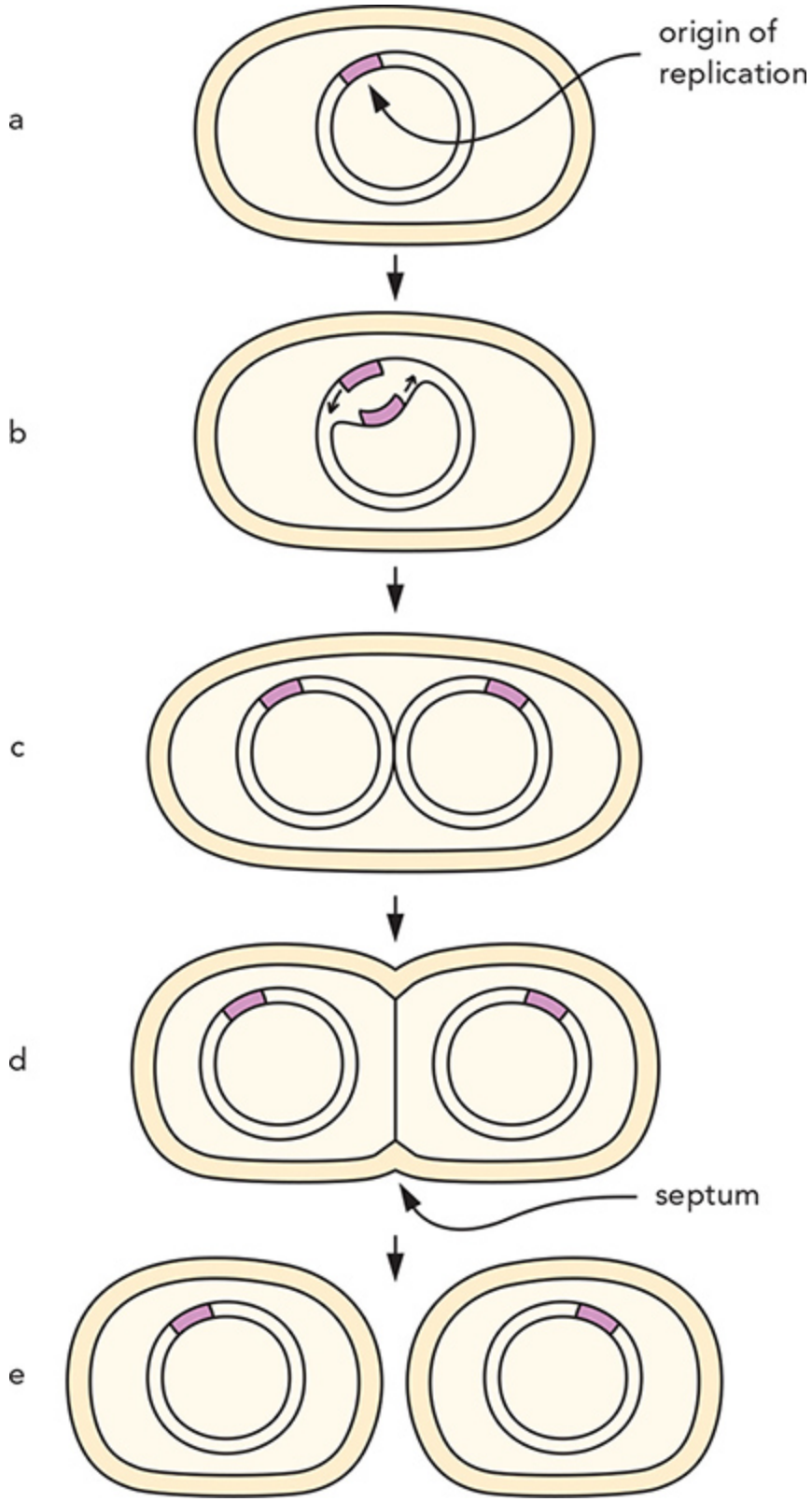
Many of you, perhaps, have read other books in the *Teaming* series or at least understand the exudate-driven model of the soil food web: plant exudates attract bacteria and fungi, which in turn attract nematodes and protozoa who consume the bacteria and fungi, releasing plant-usable nutrients that can move into plants. The same microbes move through the soil, creating structure, controlling pathogens, chelating metals, solubilizing phosphates, and conducting other activities vital to a healthy soil food web.

For our purposes, however, it is important to pause and consider saprophytic bacteria, which (along with similar fungi) are the key decomposers of organic matter in the soil. Sure, earthworms and other members of the soil food web help, but ultimately, from a plant's perspective at least, it is fungi and bacteria that take apart organics, reducing them to their simplest forms.

HOW BACTERIA EAT

Some bacteria are photosynthesizers. These make their own food just as plants do. They contain chloroplasts (or their precursors) and use the energy of the sun. Technically, photosynthesizing bacteria are autotrophs.

Most bacteria, however, are chemotrophs: they get their energy by breaking down organic and inorganic substances found in their environment. There are two kinds of chemotrophs: chemoautotrophs (those that break down inorganic molecules like ammonia or sulfides) and chemoheterotrophs or simply decomposers (those that decay organic molecules and compounds found in dead organic matter).



The process of binary fission. Bacterial chromosomes are circular (a), not rod-shaped as in cells undergoing mitosis. In binary fission, a bacterium's chromosomal circle is broken, and a duplicate is created by replication (b). The cell elongates as the two chromosomal circles move toward opposite

ends of the cell (c). In order to share the cytoplasm between the dividing cells, the membrane that surrounds the mother cell pinches into it, until a wall (more properly a septum) forms and grows, eventually splitting the bacterium into two halves (d). The missing cell walls are replaced, and the process is complete (e).

Chemotrophic bacteria derive energy through respiration, a process that occurs inside the cell. First, however, strong enzymes are released into the environment; these are designed to break down targeted foods. Then the disassembled compounds are moved through wall and membranes into the cytoplasm, where respiration occurs. Diffusion—that movement of molecules to less concentrated areas—is usually what sends these nutrient molecules on their trip to and past the cell wall. They pass through the bacterial membrane by continuing to diffuse or by using energy (active transport) or carrier proteins (passive transport).

And finally, in their quest for energy and nutrients, some bacteria enter into symbiotic relationships; these can be parasitic relationships, mutually beneficial ones, or one-sided commensalism.

BACTERIAL DECAY

Certain bacteria are well known for their role in the decay of organic matter in the soil. Even before tissue dies, leaves and flowers harbor millions and millions of bacteria, some endophytic. These attack whatever tissues they can. This is why food spoils or leaves develop necrotic spots even before plants are dead.

Once death occurs, decay accelerates. Anaerobic bacteria (*Clostridium* spp., for example) create butyric and other acids as they break down complex sugars, often creating a typical decay smell. You might smell ammonia and sulfur (derived from proteins and hydrogen sulfide) too.

And while the anaerobic bacteria do their thing, aerobic soil bacteria genera such as *Azotobacter*, *Enterobacter*, and *Pseudomonas* decay bonds and break up those molecules of life into smaller components, always accompanied by the release of lots of CO_2. The decay continues, and humus materials, resistant to further decay, are formed.

Cellulose, the major component in plant cell walls, is not easy to break down. *Cellulomonas* and *Clostridium* spp. work hard to do the job, producing lots of wall-destroying enzymes. Lignin is also extremely resistant to decay. In fact, white and brown rot bacteria are helped by insects and other animals that physically degrade lignin.

Increasing numbers of bacteria raise soil pH, and while they eat, they create CO_2 for plants to use, hormones and vitamins to help protect the plant (and sometimes feed it), and toxins to take out pathogens that could harm plants.

Last but not least, chemicals in soils (including those in pesticides) are degraded during bacterial nutrient acquisition, broken down into nontoxic constituents.

In the end, microbial noshing converts inaccessible nutrients to plant-usable forms. Nutrients are retained in the microbes' bodies instead of washing away. Bacterial activity initiates soil structure with its slime, fosters aeration, and loosens compacted soils (not to mention the other soil food web members that run around in the soil, so to speak, consuming bacteria and creating burrows and pathways usable by air and water).

BACTERIA AND SEEDS

Every seed carries bacteria from its mother plant. This is one way endophytes move from one plant generation to the next. Some seeds even have special structures that give their bacteria better protection. (They must be very important.)

Removing bacteria from seeds causes lots of problems for plants, which might come as a surprise to some gardeners. Some seeds won't germinate without their microbial hitchhikers. Other hitchhiking bacteria are fed by the seedling's exudates and populate the soil, but not if the seed has been sterilized. And removing bacteria from roots stops root hairs from forming —a death sentence to a plant.

How many bacteria might a seed carry? This would depend on the species and genotype, soil properties, and a whole range of other considerations. The high-end estimate is 100,000,000 (106 to 108 CFU/g seed). Even seeds known for tough exteriors that require digestion by a squirrel or bird (or the occasional blueberry-loving bear that ambles through my backyard) have bacteria on them, despite the acidic treatment such passage entails. In fact, seeds probably pick up bacteria while making the trip through a digestive tract. Some of these may even turn into endophytic bacteria at some point in their lives.

In general, seed bacteria end up helping the plant produce or modulate growth hormones. Some lower ethylene levels or fight off pathogens or

move into the soil, where they perform other useful services (like producing siderophores that deliver metals to—or sequester them from—plants). They may break dormancy.

BACTERIA AND ROOT HAIR GROWTH

Endophytic bacteria appear to be responsible for proper root hair development. Studies clearly show that if endophytic root bacteria are removed, seedlings do not develop root hairs. Roots are stunted and disoriented, growing haphazardly, ignoring gravity. When the bacteria are put back, root hairs develop; roots respond to gravity, and all is well with the world.

There is still much to figure out. At Rutgers, researchers removed the bacteria from rice roots. As expected, this resulted in stunted roots. When the plants were then exposed to ethylene (1 ppm), normal root growth returned. Remember, endophytic bacteria produce ethylene as they enter a root cell's periplasmic space. Hmm.

NOMENCLATURE

Just as with plants, bacteria have a naming system that separates them into hierarchical groups based on such traits as size, shape, structural characteristics, color, staining, or metabolites produced. And just as with plants, there are lots of unpronounceable words. It is very complicated to us non-scientists, so let's peek under the tent a bit.

The most useful designation is the species designation. Technically, these are organisms that can breed with each other. Plants are easy, but it is a bit more difficult with bacteria since they pass bits of DNA around, interspecies. Slight genetic variations in species results in strains of that species. Individual species of bacteria with the most similarities are assigned to genera (singular, genus). Genera are grouped into families by broader similar traits, and then into suborders, orders, classes, phyla (singular, phylum), and kingdoms. Finally (and mercifully), bacterial phyla, like all phyla, are assigned to one of three domains: Eukaryota, Archaea, and Bacteria. (Guess which one.)

What results is a catalog of bacteria. The mnemonic for the main levels in this hierarchy, from most to least broad, is the question, "Did King Phillip come over for great spaghetti?" (domain, kingdom, phylum, class,

order, family, genus, species). It is a huge catalog, however, and growing all the time as more and more bacteria are identified using genetic procedures.

Scientists talking about bacteria use the same binomial nomenclature system invented by Carl Linnaeus for plants. First, there is a generic name, always based on some meaningful encompassing word, usually derived from Latin or Greek; it always begins with a capital letter. This is followed by something specific about the organism; the specific epithet is always lowercased. Both the genus and specific epithets are italicized. No level above genus is italicized; everything else, from order to domain, is capitalized but in roman type.

PHYLA

Endophytic bacteria are often grouped into phyla in study reports. Each phylum name is uppercased (but not italicized), but that's where the commonality ends, as phylum names don't have a simple common ending. Some phylum names end with -bacteria, others with -aeota. For our further purposes, trust me: if it is really important, I will call a phylum a phylum. Assignment into a phylum is based upon the sequencing of a particular set of genes, the 16S rRNA genes.

It is increasingly easier and cheaper to do genetic analysis, and happily for scientists, the genetics of individuals in a large diverse population can now be determined relatively swiftly. Imagine taking a sample of a bustling rhizosphere; being able to report who is there—and just as important (and maybe more), who is missing—is vital.

An analysis of a typical garden soil revealed bacteria from 32 phyla, each with its own suite of interesting and distinctive bacteria. The vast majority of soil bacteria belong to the following nine phyla.

Acidobacteria. This diverse phylum is nearly ubiquitous in soil (up to 50% of populations at times). Members include *Acidobacterium capsulatum*, *Bryobacter aggregatus*, and *Holophaga foetida*. Some of these live in—wait for it—very acidic environments.

Actinobacteria. These Gram-positive bacteria decompose organic matter, making it available to plants. They are, as any gardener worth her salt knows, extremely critical to the operation of the soil food web.

Bacteroidetes. Gram-negative and able to survive in lots of different environments, from soil to the guts of animals. Often found in association with truffles.

Chloroflexi. Some members of this phylum are thermophiles, bacteria that do well at higher temperatures. These are the guys that help in your compost pile if there is enough oxygen and temperatures get high enough.

Firmicutes. These are mostly Gram-positive bacteria. Firmicutes include *Lactobacillus* (yogurt!) and our old medical friends *Staphylococcus* and *Streptococcus* (aka staph and strep).

Gemmatimonadetes. Gram-negative and beyond exclusive: last I looked, this phylum has only one genus and one species, discovered in sludge in 2003.

Planctomycetes. Gram-negative bacteria that live in freshwater, brackish water, and salt water. Remember, all plants have endophytes, even aquatic ones.

Proteobacteria. This phylum (aka purple bacteria) includes soil-dwelling nitrogen fixers as well as pathogenic bacteria (the order Legionellales; the genera *Escherichia*, *Helicobacter*, *Salmonella*). They are Gram-negative. Mitochondria descend from Proteobacteria. (Very cool.)

Verrucomicrobia. Gram-negative bacteria. Another very new phylum with, so far, few members but a lot of individuals, population-wise.

GENERA

You will come across certain genus names a lot when dealing with endophytic bacteria. Genera are groupings of species. Again, you do not have to memorize them, any more than you have to make a Baked Alaska from scratch to enjoy it. You will come to recognize many of these.

***Enterobacter*.** This genus of bacteria helps roots acquire soil nutrients and develop. Not only are its member species found in seeds, they colonize the soil around seeds, consuming much of the exudates there. This protects the emerging seedling from pathogens. All species solubilize phosphate; some fix nitrogen.

***Methylobacterium*.** This genus is often found in the phyllosphere, the area immediately around leaf surfaces. Its members are metabolizers of the methanol emitted from plant cells as a signal. By metabolizing methanol, methylobacteria may be part of the regulation system of the plant. Species reside in the xylem, seeds, and leaves. They fix nitrogen, fight pathogens, promote germination, and help plants grow by producing and stimulating hormone production.

***Pantoea*.** A genus of about 20 species of bacteria, formerly included in *Enterobacter*. They are often pathogenic; however, some produce antifungal metabolites that protect seeds.

***Pseudomonas*.** Many species in this genus are common bacteria of the rhizosphere (the soil immediately around a plant's roots) and endosphere (the plant's internal tissues). Some pseudomonads engage in the rhizophagy cycle; some suppress diseases, chelate iron, and stimulate root and aboveground plant growth. *Pseudomonas syringae* and other pseudomonads can do harm as well; they are among the best-known plant pathogens, often competing with and negating the good work of their benign siblings. On the other hand, some make water freeze at a higher temperature, thus protecting plants from freezing (to a point).

SPECIES VS STRAINS

Species look and act like each other and differ significantly from other species. They always have a tremendous number of similar characteristics, not just one. However, within any one bacterial species, there can be bacteria with slight differences. We know plasmids transfer only a few traits; the new bacteria differ from the "norm" in only small ways, but these small differences are enough to set the strain apart from others in the species. This is what heirloom tomatoes are, local strains of the same great tomato plant.

LAST CRY—FOR EDUCATIONAL REFORM

Bacteria are everywhere. Everywhere! Our own bodies have more bacteria cells than human cells. Bacteria feed plants, make us sick, keep us well, give us wine and yogurt, and so much more. Bacteria influence every single

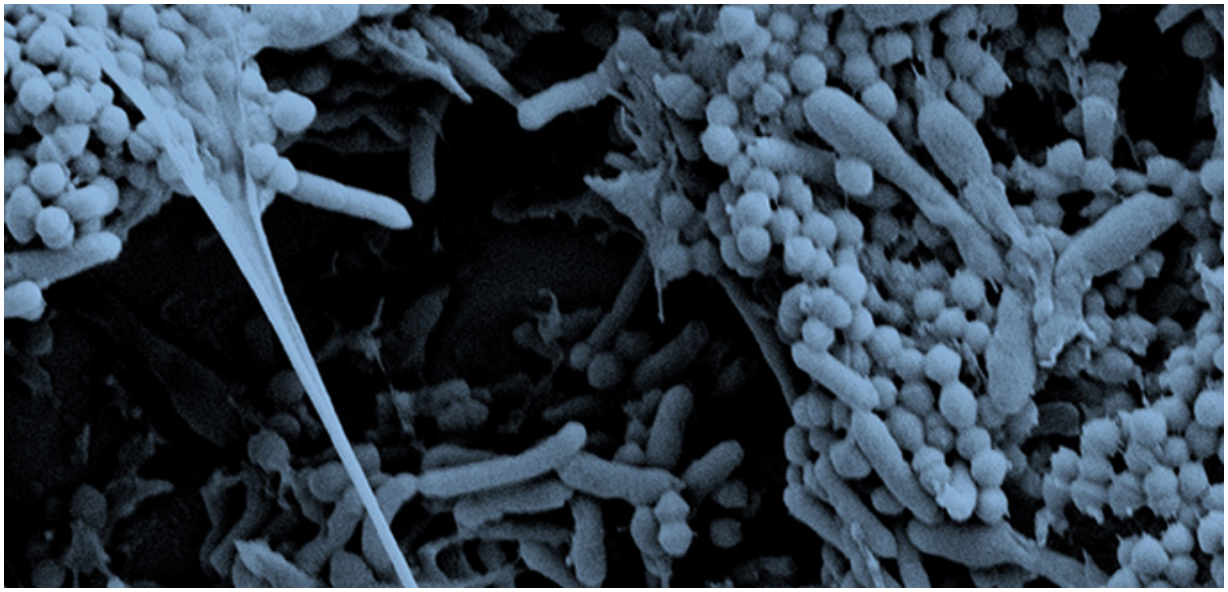
thing we do (and maybe what we think). So why don’t we appreciate them more, and why don’t we learn all about them when we are young?

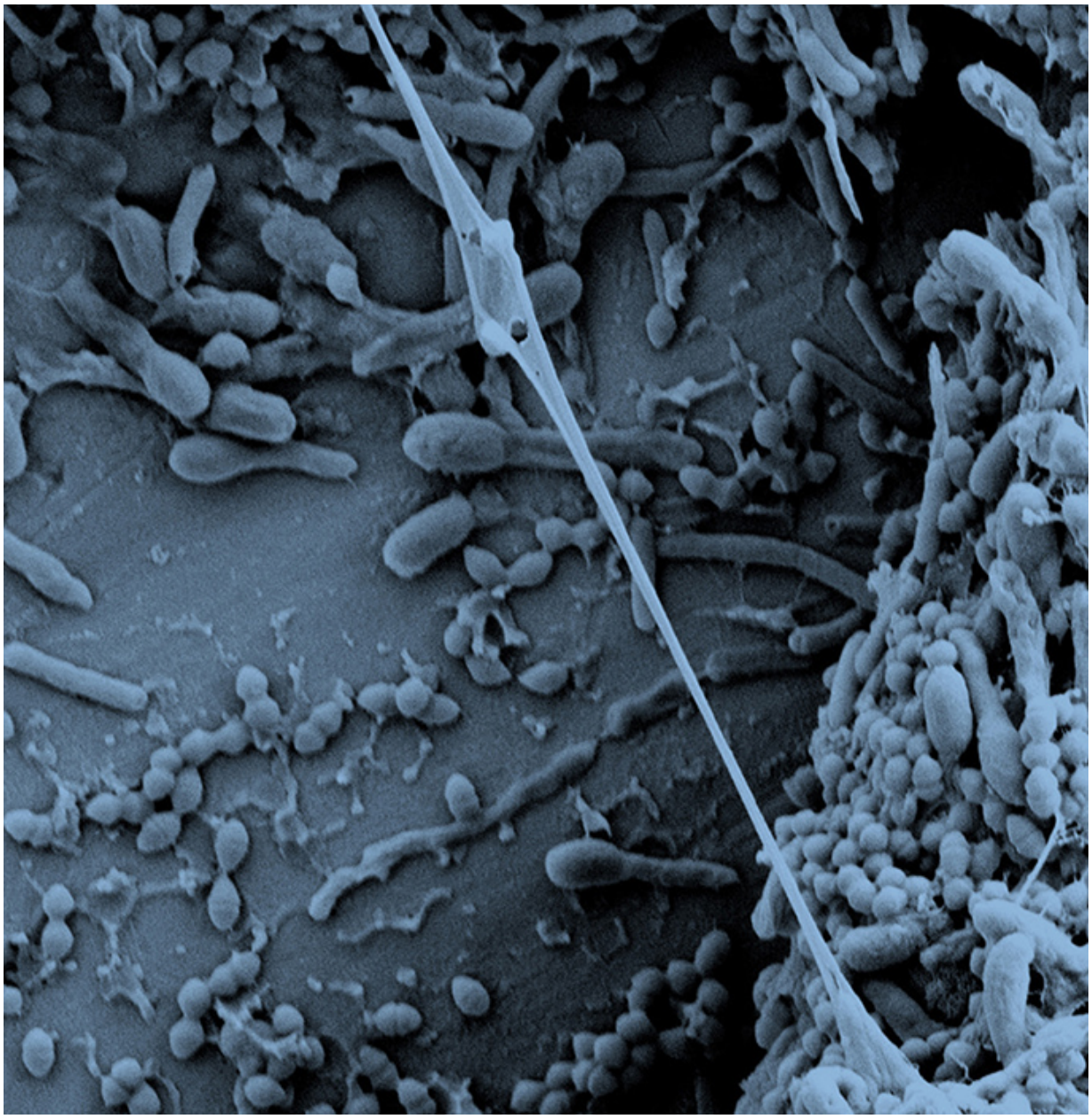
Consider that young children are taught everything about dinosaurs these days. And I mean young. Dino plush toys accompany toddlers to sleep all across North America. There are dinosaur coloring books, dinosaur cartoons, dinosaur movies, dinosaur bone museums, dinosaur class assignments, and book after picture book of dinosaurs. By the time my grandchildren were five, they knew the differences between brachiosauruses and allosauruses.

Yet children can’t name one bacterium. They wash their hands because of them. They bathe because of them. They don’t eat food dropped on the floor because of them. They are vaccinated against them. Society focuses on an extinct set of animals that don’t have a single thing to do with life as we live it (other than oil and gas), while bacteria, in the here and now, impact every single thing we do on earth. This is wrong.

You and I may have trouble with the names of endophytic bacteria, but all those complicated names of dinosaurs roll off the tongues of kindergarteners (and they can tell you how they lived, what they ate, and how many young they had). If they can spout *T. rex*, they could just as easily manage *E. coli*.

It is time to take dinosaurs out of the educational curriculum and put ubiquitous bacteria in. It doesn’t have to be complicated, and they are every bit as interesting as extinct dinos. Now, to make some pseudomonad plush toys so kids can learn about rhizophagy and endophytic bacteria.





Endophytic Bacteria

EVERY SINGLE PLANT harbors at least one microorganism that lives inside the plant for part of its life without harming it. These are endophytes (from the Greek, *endo* “within,” *phyto* “plant”). Endophytes were once thought to live between plant cells, not inside them. With better microscopy, they have been found living inside plant cells too.

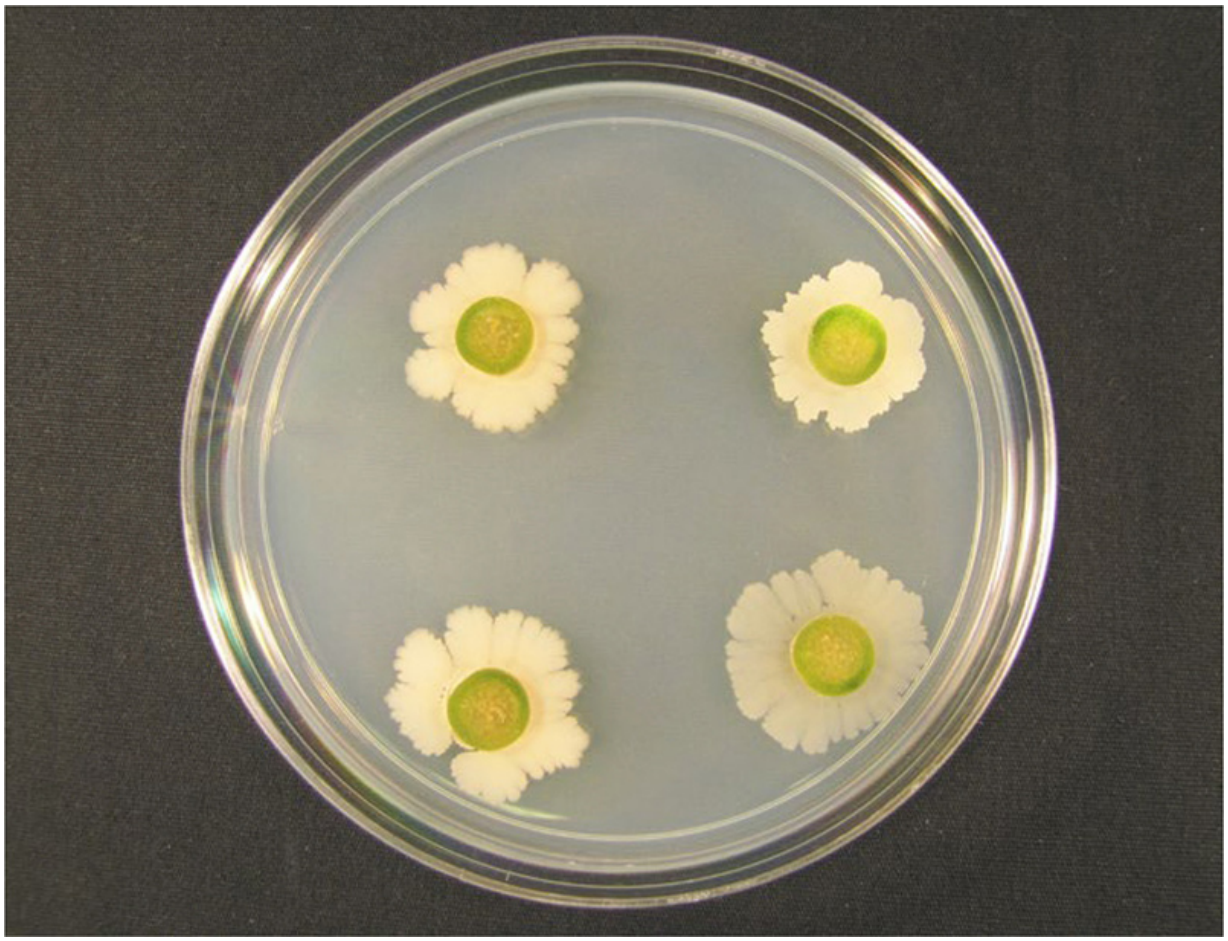
Bacterial endophytes were first described in 1809 by German botanist Heinrich Friedrich Link. Link, as did most other 19th-century botanists, concluded that these entophytae (as he called them) were parasitic. In fact, any bacterium found inside a plant was a pathogen until Dutch microbiologist Martinus Beijerinck found rhizobia in legumes in 1888. Other scientists then determined these bacteria could fix nitrogen—which was a good thing.

Still, plants were generally thought to be sterile internally—until the 1940s, when endophytes were found in pasture grasses that were causing problems with grazing cattle. Scrutiny and recognition kicked into high gear, and now over 200 different bacterial genera from 16 phyla are known to be endophytic.

Some endophytic bacteria are found exclusively in the cells of one plant part, such as around a leaf’s stomata. Others reside in various parts, from stems and flowers to seeds. They may even live inside the sacred nuclear envelope of eukaryotic cells, as their tracks have been identified on such a surface.

Most endophytic bacteria come from three phyla: Actinobacteria, Firmicutes, and Proteobacteria. Participating bacteria include a few genera that are familiar to growers: *Bacillus* and *Pseudomonas* among them. By the end of this book, you will recognize more of them.

Just to be fair (and I always try to be), other microorganisms are endophytic—mycorrhizal fungi, for instance, but many other fungi too. Archaeans (which look a lot like bacteria) also live in plants. Even algae grow symbiotically inside plant cells, though only in seaweeds, and oomycetes (fungus-like water molds) are endophytic at times. There are even endophytic insects.



Vanilla stem showing endophytic bacteria in culture.

Why should you care about endophytic bacteria? Besides aiding in nutrient acquisition, endophytic bacteria help their plant hosts fight pathogens and develop tolerances to all manner of environmental and biotic stresses. With the discovery of the rhizophagy cycle, we see they are also intracellular nutrient sources for their hosts. (They are very accommodating guests.)

WHENCE ENDOPHYTES?

A huge population of different kinds of microbes lives around roots, in the rhizosphere, and some of these enter the roots (that is, they become endophytes). So, no surprise, there is a direct correlation between the types of bacteria in the rhizospheric soil and those inside the plant.

Sure, plants get infected by aboveground bacteria—say, through a wound. These endophytes are transferred horizontally from an individual

plant to a neighboring plant. Mostly, however, a bacterium gets into a plant through its roots, whether that bacterium was delivered in the plant's seed or comes from a population already in the soil.

Seeds are great vectors for microbes, and the microbes seeds carry are usually necessary for the plants to perform optimally. Lose them, and the plant suffers. Cotton (including seeds), for example, is cleaned with an acid that kills off bacteria; the result is that growers need to apply pesticides to replicate, in part, what the lost seed bacteria would've done to protect the next generation of cotton plants. Little wonder that the cultivation of cotton results in the highest use of pesticides in the United States: the necessary endophytic bacteria—those that would protect the plants and help them grow properly—are absent.

Even if you didn't know about the soil food web (is that possible these days?), it is just logical that a diverse set of soil microorganisms around a plant would result in a higher frequency of bacterial infection of that plant. But with regard to endophytic bacteria, the plant *wants* the bacteria for the benefits they impart, and the bacteria get a safer place to live.

HOST PLANTS

All plants—from familiar agricultural crops to prairie, forest, and desert plants to aquatic plants—host endophytes. All plants, every single one. This surely implies that both the bacteria and the plants benefit from the arrangement.

Unlike mycorrhizal fungi, which are not terribly specific in choosing partners, plants are very selective about the bacteria they connect with. All manner of studies have moved endophytes from one kind of plant to another, seeking to transfer a benefit. Sometimes transplanted endophytic bacteria help the new plant exactly as they did the original host. Sometimes they hinder growth in the new host.

The central theme of growing crops using the soil food web—however you know this practice: organic gardening, biodynamics, Korean natural farming (KNF), or some other biobased system—is that plants use valuable photosynthetic energy to biosynthesize exudates to attract bacteria and fungi to the root zone. The attracted microbes make organic matter available in the inorganic form required for plant uptake. We are now learning that many of these bacteria are capable of turning endophytic—

entering plants and feeding them internally, or simply traveling to other parts of the plant to live out their days.

Exudates are one way plants communicate with bacteria. The diversity of soil populations makes it difficult to show the exact role of each exudate in signaling between plant and bacterium, but there have been enough experiments to show that it occurs. There is also good evidence that the plant calls the bacteria and not the other way around.

A lateral root releasing exudates.

Normally plants put up defenses to stop a bacterium from even landing on them, much less getting into them. Yet plants tolerate endophytic bacteria. It is logical that the plant sends the signal for bacteria to enter. How else would bacteria know the plant's defenses are down or even that a plant root was near? Nodule-forming bacteria and mycorrhizal fungi, both endophytic, are attracted by the plant's calling.

HOST COLONIZATION

Bacteria can enter a plant above ground, but roots are the main way in. The specific venues for entry are the root cap and meristematic zone (where the cells are young and the walls are thin) and root cracks and other fissures formed during the emergence of lateral roots. In such places, bacteria are able to sneak into the plant without triggering too many of its defenses. Or, more strategically, the plant evolved to tolerate the bacteria because of some benefit.

When it comes to the rhizophagy cycle, the entrance into the root system is via cells in the meristematic zone. In addition to very thin new walls, root meristem cells are in direct proximity to where exudates are released, so there is a good population of bacteria there.

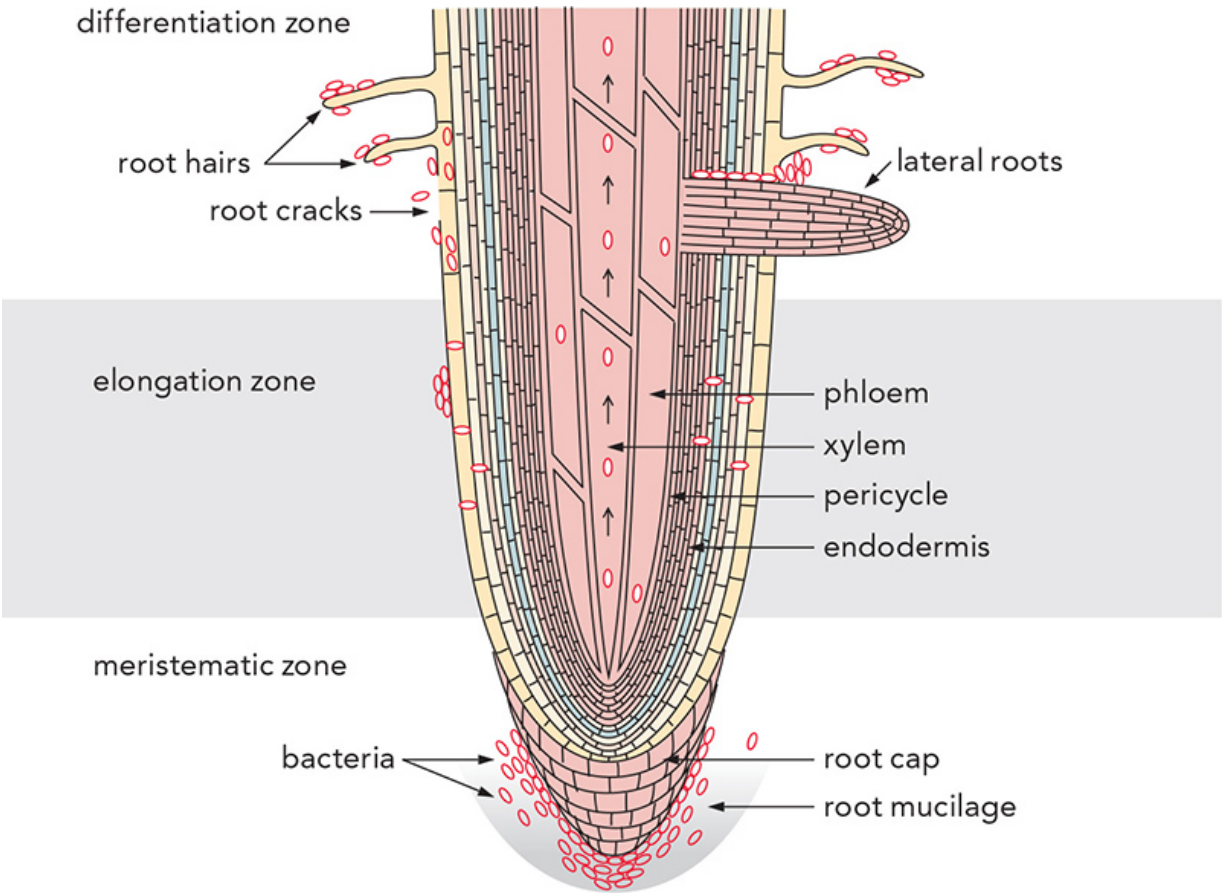
Why would a bacterium want to live inside a plant when it could live outside in exudate-rich soil? To avoid competition. There is a lot of competition for resources in the rhizosphere, including space. Clearly, it is less competitive inside the plant when it comes to space, and not only does the endosphere offer protection from the hordes, it offers nutrients as well.

So, evolutionary forces being what they are, some bacteria developed the necessary traits to get right up against those meristem cells (or the junction of root hairs or lateral roots with the main root), where they use enzymes to break in and move into the cell. Perhaps these bacteria have better mobility. It might be they have the right kinds of pili or the ability to produce a better biofilm, with the proper bacterial population, to outcompete fungal cousins. Or they could just be in the right place at the right time—finding a crack in the root and seizing the chance to move through.

Exudates ensure certain sections of the root cap surface are quickly colonized by bacteria after seed germination. But the whole root cap is never coated entirely, which suggests other factors are at play, like timing and exact location of exudation, presence of mucilage, the quality of bacterial attachment, competition, the local pH, or how large, area-wise, a bacteria population will grow. It could be that complete coverage would interfere with adsorption of water and nutrients, which still occurs even with the rhizophagy cycle.

How exactly do these bacteria get inside a plant that normally defends itself from infection by bacteria? First, the exudates bring the bacteria to the rhizoplane (the surface of the root). There is even evidence that some plants create the illusion of an empty bacterial slime colony, ready for the taking, in order to attract bacteria to the root surface. When triggered, some of

these bacteria break apart cellulose and pectin in the meristem cell wall using enzymes that attack the cellulose and the waxy substances in it.



How endophytes get into plants: points of entry and other wayfinding landmarks.

But why aren't the plant's defenses triggered? Maybe endophytic bacteria keep below a triggering population threshold; or perhaps they use one or more phytohormones to moderate any plant defense. Perhaps the plant doesn't put up a defense at all because the endophytic bacteria have evolved to use lower concentrations of wall-degrading enzymes than their pathogenic relatives and so are not detected. Remember, the plant signaled for the bacteria to come to the rhizoplane in the first place, using exudates; it must have evolved to purposely ignore or even promote the entry of certain of these, thanks to "benefits received."

LIFE ON THE INSIDE

Most endophytic bacteria remain close to where they enter a plant—for example, a fissure created during formation of lateral roots. Some move

into the apoplastic (intercellular) spaces between plant cell walls; these walls are loaded with water and organic nutrients, amino acids, and carbohydrates, which the endophyte guest can use to support itself. Once in, endophytic bacteria head anywhere their little flagella can take them, even to entering the central core of the plant that holds the xylem and phloem. An endophytic microbe can move throughout the plant via the connected cell walls and never enter the periplasm or the cytoplasm.

Many free-living soil bacteria that take part in the rhizophagy cycle are part-time endophytes. These include *Bacillus subtilis*, *Pantoea agglomerans*, and *Pseudomonas fluorescens*. The key thing is when any of these bacteria pass through the meristem cell wall and into the periplasmic space, they rub up against the inner cell membrane, causing oxidizing agents (i.e., ROS) to be released from structures embedded in the cell's membrane. These degrade the walls of the invading bacteria but do not kill them. As the bacteria are transformed into (wall-less) L-forms, the dissolving walls become plant-usable nutrients that are transferred into the cytoplasm.

SPECIAL DELIVERY

Some endophytic microbes enter a plant's reproductive system (flowers) and are trapped inside seeds that form there. Or, put another way, seeds receive a microbiome from their mothers—another reason gardeners should treat seeds properly. If seeds are happy, seed bacteria are happy.

The bacterial mix transferred by a seed is almost certainly specific to that variety of seed. A great study showed this using corn (*Zea mays*). The early ancestor of corn is a grass, teosinte. Ancient teosinte from Mexico has been bred into modern corn all over North America. The corn we and our livestock eat (including my favorite, popcorn) is the product of generation after generation of breeding and crossbreeding. The study revealed that early teosinte had seeds with specific endophytic bacteria. Amazingly, all the different modern versions of corn still have the same core group of endophytes, even after all that breeding: *Clostridium*, *Enterobacter*, *Methylobacterium*, *Paenibacillus*, *Pantoea*, and *Pseudomonas* spp. were present across the board, right down through the continuum.

Of course, as corn seed was traded throughout North America, corn endophytes evolved so that they could thrive in new locations, but the same

or very similar sets of bacteria after all these years? Given how Nature works, this has to be because both plant and bacteria benefit.

Endophytes carried by seeds end up in the soil. At least one endophyte isolated from maize during the study established colonies in the rhizosphere, not just in the corn plant. Is this a result of the rhizophagy cycle? Could it be these formerly endophytic bacteria need a free-living state to suck up nutrients to take with them on their next rhizophagy trip in the plant?

Another study showed that seedlings of tall fescue (*Lolium arundinaceum*) grown with seed containing endophytic bacteria had 30% more nitrogen than control seedlings grown without the same microbes. Do these bacteria use siderophores and nutrient-acquisition metabolites to fatten up so they can make their plant host happy? Is rhizophagy involved?

Lots of research projects are studying the role of endophytic bacteria in the rhizophagy cycle. In one, seeds were cleaned of microbes and then grown alongside uncleaned controls. The sterile seeds developed weak and disorganized root systems, including a loss of root gravitropism. The seedling's roots often grew sideways and even on the soil surface and upward. Roots that did develop were weak. When the bacteria were put back into the system, lo and behold, roots returned to normal growth.

Next, seed-borne bacteria clearly influence root elongation, branching, and development of root hairs. In fact, sterilized seeds produce seedlings that lack root hairs entirely. Clearly something is going on—something, perhaps, to do with the production of auxin and cytokinins, phytohormones that are important to root development.

MUTUAL BENEFITS

Considering it is such a competitive world "out there," why *wouldn't* bacteria become endophytic? In the soil, multitudes of enemies and fluctuations of pH, moisture, and nutrient composition (among other factors) make for a difficult, energy-expending life. Inside a plant, these problems disappear; there is protected space and a source of food. And on the flip side, some microbes need the host partner in order to function properly.

Endophytic bacteria benefit their host in several ways. First, they can assist in obtaining nutrients. Second, they are capable of stimulating growth, apart from nutritionally. And third, endophytic bacteria can greatly

increase host resistance to stresses, both biological and environmental. From the plant's perspective, these are pretty good rewards for simply allowing bacteria to live where it's safe and glean some scraps of food. In fact, they sound like things any gardener or grower would want to take advantage of.

Some endophytes produce chemicals that trigger plant growth; others produce chemicals that slow growth. Some can fix nitrogen, and in return for the safe home, complete with oxygen and a fully stocked refrigerator, these bacteria share some fixed nitrogen with the plant, to its betterment, and live the happiest of lives (as long as the plant does too). Others solubilize phosphate, sequester iron, fight fungi, defend against pathogens, or produce ethylene, auxin, and other hormones. This laundry list of goodness is why we need to learn about these endophytes.

NITROGEN FIXATION

Nitrogen fixation makes atmospheric nitrogen (N_2) usable to plants by converting it into ammonia (NH_3). The fixed nitrogen, in ammonium form (NH_4^+), remains in the soil unless it is transformed chemically or microbiologically into nitrates (NO_3^-). The bacteria that accomplish this feat can be free-living or endophytic. They can reside in nodules, as is the case with rhizobia bacteria in legumes, or they can fix nitrogen without housing.

Nitrogen fixation is no minor matter. Plants need nitrogen more than any other essential element. Usually plants get it from the soil, where it has been fixed by free-living bacteria; however, endophytic bacteria can fix nitrogen right inside the plant, a real advantage.

Replicating the nitrogen-fixing abilities of endophytic microbes in non-legumes has become a Holy Grail for the fertilizer industry. Synthetic fertilizers (which cause so much environmental damage) are energy-demanding, climate-changing, and inefficient. Finding a better way to get nitrogen to plants is a big deal, worth billions (and yet Nature has been doing it for free for eons). One study found endophytic bacteria supply as much as 47% of all the atmospheric-derived nitrogen in plants and as much as 70% of the nitrogen their plant host needs. That is a lot of free fertilizer. If you could increase these quantities or trigger the process more often in non-legume plants, imagine how much better the environment would fare.

Nitrogen-fixing bacteria also participate in the rhizophagy cycle; however, the nitrogen produced during this phase appears to be limited to what is stripped out of the cell wall by the reactive oxygen treatment.

The bottom line is that some endophytic bacteria fix nitrogen inside the plant host and some literally become nitrogen inside the plant. Nitrogen in constant and ready supply makes for a healthy plant, one that grows better than similarly situated plants without endophytes. No wonder a plant agrees to Airbnb-host nitrogen-fixing bacteria.

PHOSPHATE SOLUBILIZATION

Next to nitrogen, phosphorus is the nutrient most needed for plant growth. Phosphorus supplies are finite, however, and we are rapidly using up our planet's limited stores. This being the case, it makes sense to study the role endophytic bacteria have in supplying phosphorus to plants.

Chemically, phosphorus readily combines with other elements and becomes locked up, difficult for plants to access. Some soil bacteria use enzymes to solubilize (break up) phosphates, making them available; these are plant growth-promoting bacteria (PGPB)—remember that acronym!—and they are often endophytic.

Endophytic bacteria produce auxin, causing increases in root growth and branching, resulting in more root mass. This increased mass provides more surface area for uptake of phosphorus. And more roots increases exudates that attract more rhizospheric PGPBs, which in turn can solubilize more phosphates.

SCAVAGING SIDEROPHORES

Siderophores are special charged molecules, produced by bacteria, that literally scavenge their environments for iron and other metals. Plants can produce them too, in response to low levels of iron in the soil. (What can't a plant do?)

Iron is key to making DNA and needed to transport electrons during cellular energy production (I will spare you the details), so it is essential to life. Iron is also the most abundant element on earth, and you'd think a plant would have no problem accessing some. However, in soils iron is tied up as ferric iron, which you know as rust.

Both plants and bacteria produce siderophores to access iron. Notable bacteria genera that do so include *Azotobacter*, *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Rhizobium*. Their affinity with iron reminds me of little bits of iron that coalesce on a magnet. This collecting of iron is chelation. The siderophore's chelated iron (or other metal) is then moved across the membrane(s) of plants or bacteria.

Siderophore activity is described as scavenging. No wonder: soil siderophores are so attracted to iron that they are capable of venturing out beyond the rhizosphere to get it. Bacterial siderophores complexed with iron enter the root cell and release it when those ROS get to work on the bacterium's wall. Of note: siderophores can scavenge iron from plant pathogens and thus protect the host.

One reason a plant might support siderophore-producing bacteria endophytically is the great competition for metals. These have to be shared among the rest of the rhizospheric population, which seeks them too. Inside a plant, however, siderophore-carrying bacteria need share only with their host—and what's more, a host that has an incentive to keep the bacteria alive, which the competition lacks.

Siderophores also help plants tolerate living in soils with high amounts of heavy metals. These are chelated in the soil, where they remain sequestered. All is well.

GROWTH STIMULATION

The right endophyte can improve plant growth in general, but it is impossible to attribute better growth to the presence of one or more specific endophytes unless you know what and where they are. All manner of scientific developments help scientists answer these what-and-where questions, enabling them to strip endophytes out of plants and reestablish them in other plants grown sterile and microbe-free. Better microscopy, for example, is a real game changer, and nothing helps more than the ability to do DNA and RNA sequencing for identification.

What *is* definitively known is that endophytes biosynthesize phytohormones that influence and modulate plant growth. All nine categories of phytohormones (abscisic acid, auxin, brassinosteroids, cytokinins, ethylene, gibberellins, jasmonic acids, salicylates, and strigolactones) are important to plants. Some of these plant growth-promoting hormones can be synthesized economically and are already in

commercial use, and the race is on to figure out how to produce the rest. The potential is obvious: helping plants grow from within by using endophytes is a huge and as yet largely untapped market.

Here is a little more about some of the phytohormones these tiniest of critters bring to the table.

auxin. Most gardeners have heard of auxin, but few know its real name: indole-3-acetic acid (IAA). It was the first plant growth-promoting hormone to be identified; its name is derived from the Greek "to increase." It is not only the main regulator of root growth, it impacts leaf drop (abscission), flower development, and fruit production. Charles Darwin and his son, who discovered and described auxin, knew it promoted cell growth and division, but they probably had no idea that auxin can travel cell to cell, entering and exiting plasma membranes by both active and passive means. It can also be transported in the phloem and moved around the plant. The incredible thing is that bacteria seem to use it exclusively to promote plant growth.

cytokinins. Cytokinins impact a number of different plant processes, including cell division, chloroplast development, and flower and leaf drop. They can upregulate specific genes to speed growth, and they can stunt plant growth as well. Cytokinins also impact some pathogens.

ethylene. Ethylene is a gas made up of hydrogen and carbon atoms. Fungi produce this hydrocarbon, as do bacteria, which utilize ethylene to manipulate their hosts. And when things become stressful—say from wind, drought, or high salinity—plants produce ethylene. In the case of wind, ethylene makes thicker cell walls, resulting in a plant's being better able to stand up to hard breezes. Ethylene triggers flower, leaf, and fruit drop, and too much of it can cause the death of plant cells. Most important for the rhizophagy cycle, it affects cell elongation, growth, and shape.

gibberellins. Gibberellins are acidic hormones produced by some fungi and bacteria. They regulate seed germination and plant growth in several ways. I won an award for an 8th-grade science fair project on the elongation of coleus after application of gibberellic acid, a novel substance at the time. Forty years later, gibberellin experiments were at the top of my daughter's 8th-grade teacher's "trite experiments" list. Now we know bacteria use the signaling capabilities of gibberellins to manipulate their plant hosts.

jasmonic acids. Jasmonic acids (aka jasmonates) are lipid-based, signaling molecules produced by plants, bacteria, and some fungi. They can inhibit growth and have been shown to protect plants from diseases. They also play a role in fragrance (think jasmine).

salicylates. Rhizobacteria and other PGPBs are capable of producing significant amounts of salicylates, which play a role in siderophore synthesis or release: production of salicylates is often seen where iron-seeking siderophores are working. Salicylates increase plant resistance to stress and pathogens, just as aspirin (salicylic acid)—the world's most widely used drug, first derived from the bark of willows—cures your aches and pains. Who knew endophytic bacteria were such biochemists? (Not this gardener, that is for sure.) We should better appreciate their pharmaceutical abilities to assist plants.

ROOT DEVELOPMENT

It makes sense that bacteria that produce phytohormones and reside inside root cells would have at least a minor impact on the development of that root. It turns out their impact is major. Start with the presence of bacterial endophytes that speed up germination and seedling growth, and go on from there.

We know germinated seeds that lack bacteria develop weak and disorganized root systems, if they grow at all. Reinfecting seeds restores normal growth. Seed microbes also influence root elongation, branching, and the development of root hairs during the rhizophagy cycle. Seedlings from sterilized seeds lack root hairs entirely.

Root development has everything to do with nutrient uptake in plants. And sure enough, seedlings from unsterilized seeds, which still have endophytic bacteria, take up more nitrogen (as much as 30% more, according to one study). Practices which impact endophytic bacteria in seeds should be more closely examined.

IMPROVED STRESS TOLERANCE

Endophytic bacteria help plants adapt to stresses, bestowing plants with increased tolerances of drought, high salinity, temperature extremes, periodic nutrient deficiencies, wind, heavy metals, herbivory (grazing), and

pathogens. All these endophyte benefits have commercial potential, which is why there are now so many studies of endophytic bacteria, at the graduate and postgraduate levels and beyond. Hardly a week goes by without a discovery. Pay attention and look for them.

Abiotic (non-biological) stresses are caused by environmental factors like too much light or lack of oxygen. Such stresses trigger the production of ROS, damage DNA, break apart (denature) proteins, and destroy lipid membranes, particularly those of chloroplasts. Once rhizophagy bacteria are inside a root cell, the same defensive production of ROS is triggered. The problem with this protective maneuver is that it can backfire—ROS can destroy plant walls too.

Fortunately, plants have evolved techniques to ensure they remain undamaged by their use of ROS. Specifically, they produce and utilize antioxidants, which weaken or neutralize ROS. It has been impossible to fully quantify the role of this defensive use of specialized ROS, superoxide (O_2-), but it is clear a plant develops resistance to it precisely because it must protect its own cell walls. The buildup of tolerance by the plant to its own ROS helps it to deal more effectively with ROS during other times of stress. Note, however, that this is a dual-edged trowel. Endophytes can develop resistance to ROS too, and once that happens, they become difficult for the plant to control and can act as pathogens.

But back to the good. A good barrier can block a stressful invasion by bad-guy microbes, fungal or bacterial. Some endophytic bacteria enter the intercellular space and cause cells walls to thicken, which makes it more difficult for bad guys to gain entry. Others trigger the production of pectin and like substances, which make cell walls more impervious to pathogens. The thicker walls thus created are for immediate protection, but they remain in place for later use.

Some root bacterial endophytes inhibit pathogenic fungi out in the soil, around the host plant’s roots. They go out from plants into the soil and literally eat these fungi, neutralizing them and returning the nutrients to the plant. Pseudomonads (*Pseudomonas* spp.) are known to produce pyoluteorin, pyrrolnitrin, and other antifungal antibiotics, and certain endophytic *Bacillus* spp. make lipopeptides (antifungal proteins). In order to enter plant roots, endophytes need to break apart the cell walls in the root epidermis. The same enzymes they use to hydrolyze the plant’s cell wall

(chitinases, cellulases, hemicellulases, and glucanases) can also be used to destroy the cell walls of pathogenic fungi.

Halophytes, plants that live in environments of high salinity, have special endophytic bacteria with important off-label uses. These endophytes increase even a non-halophyte's tolerance of salt—and important benefit given the increasing salinity of agricultural soils—and can be applied to plants revegetating areas of high salt content. Some help plant roots accumulate sodium (NA+), so it can be extracted from soils where it has accumulated.

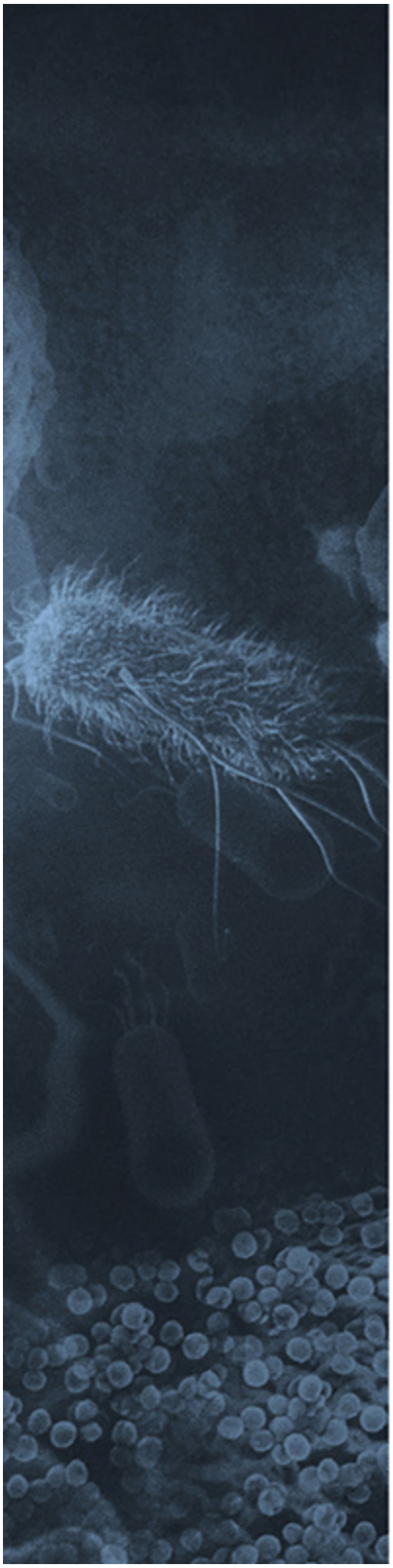
And how about this for stress relief? One pseudomonad, *Pseudomonas syringae*, can raise the freezing point of water, meaning that it may one day be possible to grow pineapples in Anchorage, Alaska. (I can't wait.)

OUTCOMPETING COMPETITOR PLANTS

Introducing endophytes while establishing or renovating a pasture can reduce weeds. This is because some endophytes in grasses prevent germination of weed seeds from surrounding plants, thus reducing competition. For example, some endophytes isolated from grasses and put on dandelions (*Taraxacum officinale*) suppressed the dandelions' growth under lab conditions. This has not been duplicated successfully in the field; however, you can be sure the concept is being worked on. There is a fortune to be made for any method that eradicates dandelions. One is coming, and now that you know endophytes, you know how it might work.

DOMINANT GENERA

Some of the most dominant bacterial endophytes among reported genera in plants—*Bacillus*, *Klebsiella*, *Pseudomonas*, *Rhizobium*—should be familiar to you by now (or, at the very least, you are starting to recognize them as bacteria, not plants). You don't need to remember their names; you just need to know endophytic bacteria do great things for plants—and that their potential for updating our agricultural and growing systems is limitless and (if you will pardon the pun) growing.



The Rhizophagy Cycle

UNTIL THE DISCOVERY of the rhizophagy cycle, the story of how plants get their nutrients looked like this: plant exudates attract and support particular soil bacteria and fungi; these in turn attract and are eaten by nematodes and protozoa; the resultant waste products are nutrients in plant-usable form. This soil food web model, codified in the USDA's Soil Biology Primer (nrcs.usda.gov/wps/portal/nrcs/main/soils/health/biology), answered a lot of questions for gardeners (such as why rototilling and sterilizing soil are bad practices) and changed gardeners' understanding of organics.

It turns out, however, that there is more to the story. Plants also eat bacteria and some yeast-type fungi for nutrients. This is rhizophagy. It was going on when the soil food web was discovered, but it took new microscopic equipment—confocal laser scanning microscopy (CLSM)—to see rhizophagy in action. Time to revisit how the soil food web operates.

A NEW MODEL

The previous version of the soil food web portrayed plants as farmers. They prepare soil with exudates, grow bacterial and fungal populations, and wait for nematode and protozoal activity to release nutrients contained in the bacteria and fungi. The plants then take up this harvest of nutrients from the soil.

Under the new model of the soil food web, plants still farm the soil, but they maintain flocks of bacteria too. Theses are rounded up and corralled just inside root meristem cell walls. There, the bacterial walls are shorn off and absorbed by the plant as nutrients. In response, the bacteria produce nitric oxide to protect themselves; this is a further boon, supplying the plant with nitrogen. Then the plant causes the bacteria to multiply. Finally, the plant grows a root hair and puts the flock of bacteria back out to pasture to get ready for the next season's shearing.

Farmer Plant Root, exuding exudates.

Ah, but there is more. The wall-less bacteria graze in the soil, regrow their walls, and continue to multiply. Once they fatten up, the plant rounds them up again, and back into the meristem corrals they go—for another shearing and harvest of nitrogen.

A single trip by a bacterium—into a root meristem cell and out through a newly formed root hair—takes a couple of days or so, during which time the plant obtains some of its nutrients by eating parts of these nitrogen-producing bacteria, intracellularly. These internally derived nutrients supplement the nutrient ions that diffuse into the plant from the soil, as per the traditional soil food web model.

The plant isn’t the only one that benefits from this newly described rhizophagy cycle. The bacteria get a boost in population, and maintaining teeming populations are what bacteria are all about. Of course, the plant

also ends up with more bacteria out in the soil to tap for nutrients, via soil diffusion and the rhizophagy cycle.

Plants, then, are bacterivores. And they consume many different kinds of bacteria, which makes sense given the diversity and numbers of bacteria in the rhizosphere. Very often one plant cell will cycle more than one kind of bacteria.

This is a good time to reemphasize the difference between the plural (many bacteria) and the singular (a single bacterium). It is natural to visualize a bacterium (neatly labeled), starting through the rhizophagy cycle by itself and then multiplying until there are enough to be released back into the soil. That is not how it works. It isn't one bacterium that enters a plant meristem cell, there are lots, hundreds. Think people in a public pool on a nice summer's day, not a lone kid or two in a backyard splash pad.

SURPRISE!

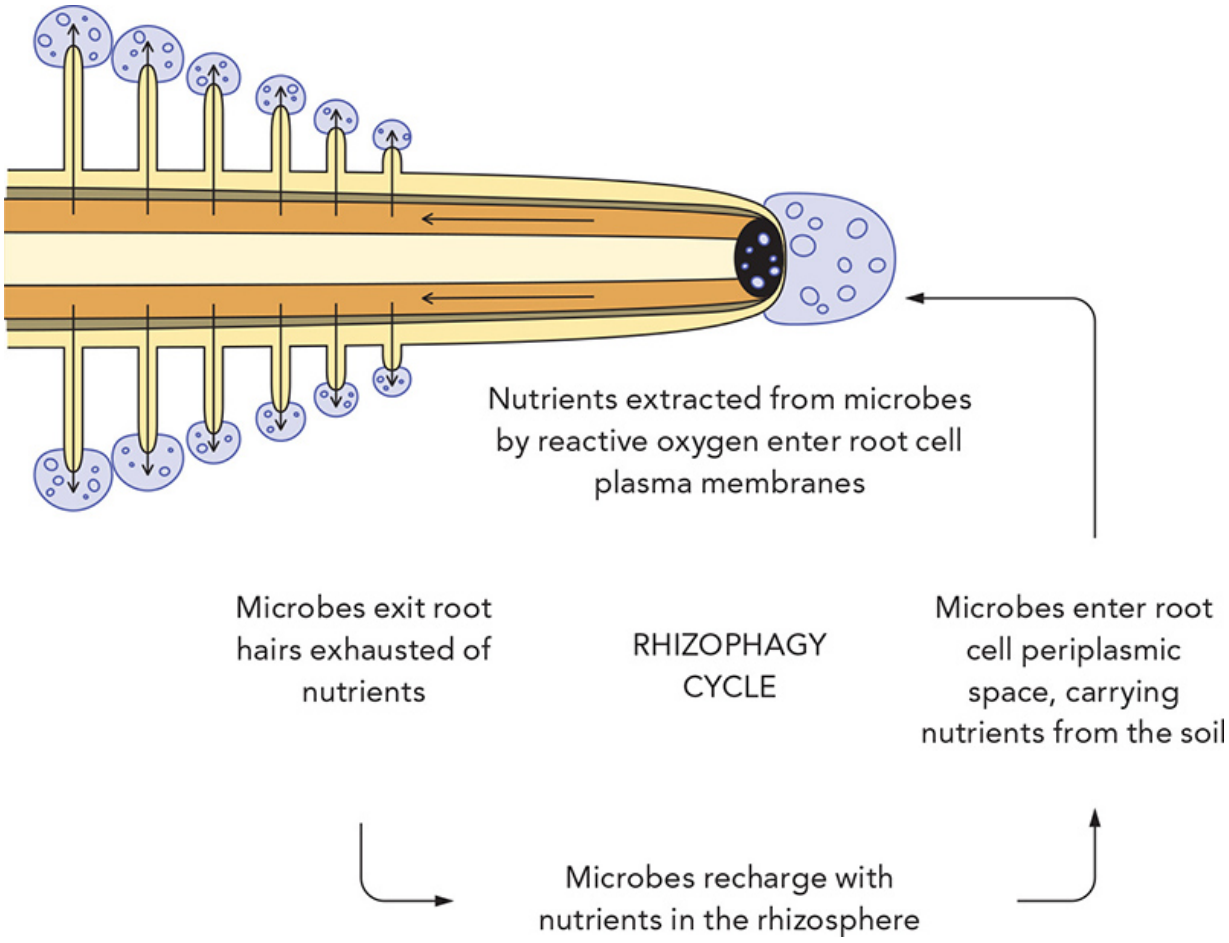
The notion of bacteria entering a plant, only to be eaten, is surprising, but why? Fungi enter plant roots. Why wouldn't bacteria do what their mycorrhizal cousins do? And just as they do outside in the soil, why wouldn't some bacteria be involved in feeding plants endophytically as well?

Many of us have been using endophytic bacteria that feed plants internally for years. We roll legume seeds in nitrogen-fixing rhizobia and seeds in the appropriate mycorrhizal fungi. We use compost full of bacteria and apply bacteria-laden aerated compost teas. So why does the existence of the rhizophagy cycle surprise us?

After all, the nitrogen supplied by rhizophagy bacteria is sort of like that supplied by rhizobia bacteria, only there is no nodule. Similar nitrogen-fixing relationships occur between *Frankia* (an actinomycete genus) and trees and shrubs in several families—birch (Betulaceae), oleaster (Elaeagnaceae), oaks and beeches (Fagaceae), bayberry (Myricaceae), and roses (Rosaceae) in particular. These plants could not survive without the nitrogen supplied by the actinomycetes in root nodules. Rhizophagy supplies nitrogen—but skips the hassle of nodulation.

Rhizobia symbiotic relationships are long-term, however; once rhizobia and *Frankia* spp. locate in their nodules, they remain for life. The rhizophagy cycle is different: participating bacteria alternate between being free-living and endophytic. This raises a question: which system operated

first, the production of food in the soil or rhizophagy? And how are these two processes related? After all, they use the same bacteria. In fact, some of them might even feed the plant via rhizophagy and then end up in the soil, feeding the plant as part of the traditional soil food web model.



A NEW TERM

Rhizophagy means “root-eating.” The word was coined in 2008 by Chanyarat Paungfoo-Lonhienne and her team at the University of Queensland. Working with roots of mouse-ear cress (*Arabidopsis thaliana*) and tomatoes (*Solanum lycopersicum*), they noticed roots took up the bacterium *Escherichia coli* and a yeast (*Saccharomyces cerevisiae*) and held them inside root meristem cells, where they were partially digested.

We now know more about rhizophagy—but not everything. The phenomenon the Australian team observed and named turned out to be cyclical and, apparently, critical for the growth of most plants, at least those

that produce root hairs. They really stumbled on something fantastic, down under, and rhizophagy is now being studied all around the world.

Again, in simplest terms, many different kinds of free-living soil microbes enter root meristem cells. There, once their cell walls are removed, they produce nutrients, multiply, form a root hair, and are expelled into new soil. They then regrow their cell walls, pick up new nutrients, and repeat the journey. (And what a journey it is.)

THE CANDIDATE POOL

A plant's exudate mix is the place to begin any discussion of the rhizophagy cycle. The mix attracts a pool of microbes to the root. The microbes travel in and around and even out of the rhizosphere, gathering nutrients and often the exudates themselves, all the while seeking to increase their populations by outcompeting their neighbors. While doing so, they form soil structure, exude metabolites of their own, and keep neighboring populations in check (all the soil food web things we learned about in *Teaming with Microbes*).

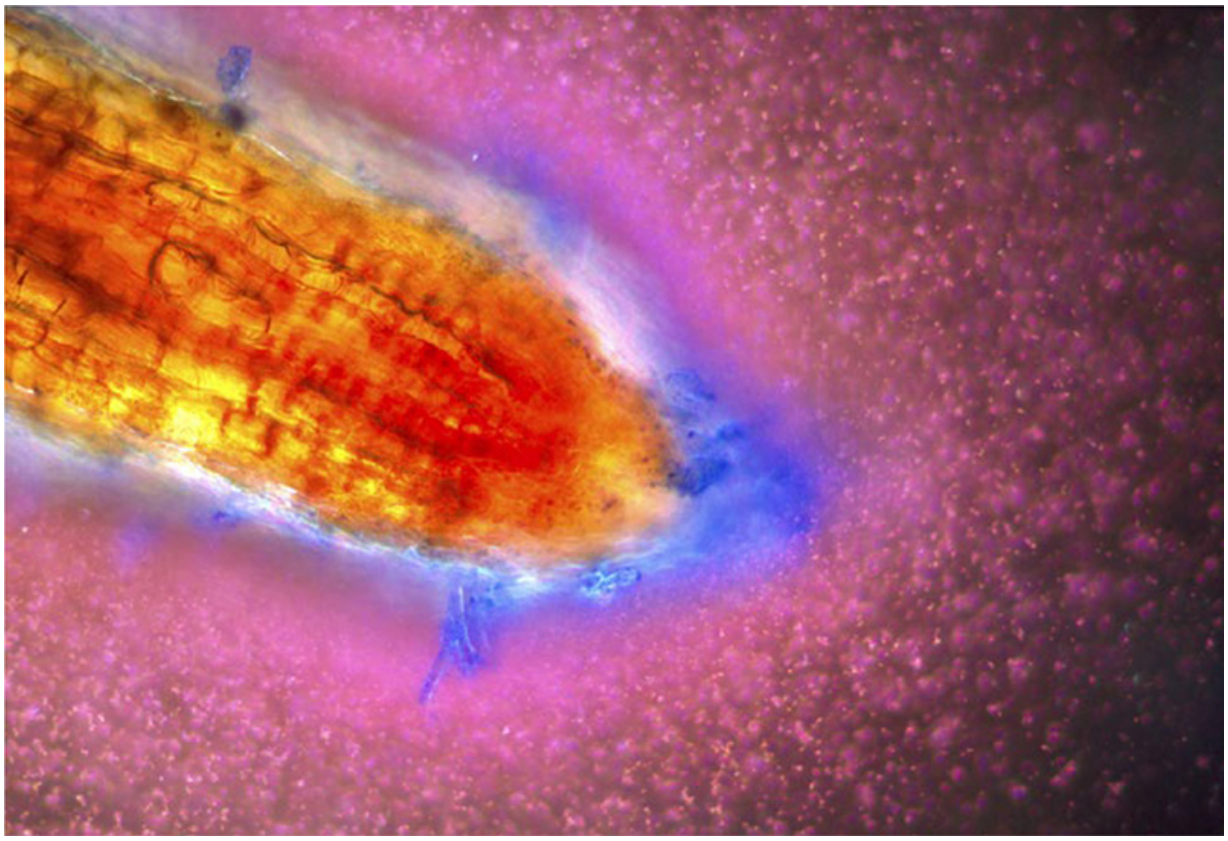
Some rhizospheric bacteria live in a biofilm attached to the root cap; these will become endophytic as they enter the rhizophagy cycle. The plant initiates the formation of a biofilm at each root cap, signaling bacteria to its rhizoplane by using special organic acids, which create a mirage of a nutrient-filled biofilm in need of tenants. Bacteria move to this spot, and a real biofilm forms and grows. The plant now has a supply of rhizophagy bacteria living right where it needs it, on the root surface.

HOUSTON, WE HAVE ENTRY

The Australian discovery team proposed that rhizophagy bacteria move into the plant cell via endocytosis—a process whereby a cell's phospholipid membrane opens, surrounds, and engulfs an object (here, bacteria) and then closes, entrapping it inside the membrane. But further developments have challenged that proposition. Let's pull together a bunch of things we have covered to understand what subsequent research suggests is a better model of how rhizophagy bacteria get inside. To do so, let's go back to that biofilm on the root rhizoplane.

Anaerobic bacteria in the biofilm produce short-chain fatty acids, including those smelly butyric and propionic acids we learned about in the biochemistry chapter. Their presence in the biofilm keeps bacterial

populations in check. Notably, if production of these acids drops in a biofilm, the population of bacteria increases. These acids probably ensure the bacterial population doesn't outstrip the nutrient supply.



A diverse mix of exudates attracts an equally diverse mix of bacteria to the rhizosphere.

As an illustrative aside, this smelly acid phenom appears to be true of bacterial colonies everywhere, even in the human gut. There, bacterial colonies are separated from the lining of the colon by biofilms. If concentrations of butyric and propionic acids drop, the bacteria leave their biofilm colonies and attack the intestinal lining. (Ouch. This causes inflammation. Crohn's, anyone?). Since we are here, once bacteria do invade, there is a defensive response in the intestinal lining—namely, the production of hydrogen peroxide (which is a weak ROS).

Plant membranes react to invading bacteria the same way intestinal membranes do: they produce ROS. Soil food webbies often compare the soil to the stomach. Here is another reason to continue to do so, but we are slightly ahead of ourselves.

Back to plant roots and their biofilm colonies. New thinking has it that some of the fatty acids in those root rhizoplane bacterial colonies are absorbed by the meristem cells on which the biofilms grow—which lowers a colony's concentrations of (regulating) fatty acids (butyric and propionic). In any case, when this happens, some of the colony's bacteria move out of the surface colony and into the meristem cells to find new supplies of fatty acids. Now we have intracellular endophytic bacteria and the start of the rhizophagy cycle.

TAKE DOWN THE WALLS

The next step in the rhizophagy cycle is the breakdown of the intracellular bacteria's cell walls. (Stick with me here.) The bacteria enter the plant cell, producing ethylene. Ethylene is a plant hormone; it can cause plant cells to elongate, but too much causes cell death. The plant cells start elongating, but the large molecules of NADPH oxidases embedded in the plant cell membrane react and produce that specialized ROS, superoxide. This is a defensive move on the plant's part, often triggered by stresses—in this case, the ethylene gas being generated by the invading bacteria.

The superoxide oxidizes the bacterial cell walls, breaking them apart. This is a delicate situation: if too much superoxide is produced, the plant's own cell walls will break apart. So, just enough is made to remove the bacterium's wall without harming the host cell's wall; however, the host may sacrifice a small bit of carbohydrates from its wall to keep its intracellular guests alive and to make exudates.

Bacteria use one of those plant exudates, arginine, and their own enzymes to make ethylene. It appears the plant provides just enough arginine to the bacteria to keep ethylene at a level that initiates root cell elongation without triggering too much growth or triggering overproduction of ROS, both of which would be harmful to the plant.

To clarify the ROS-triggering role of the microbial ethylene, scientists tested to see what happens when its formation is prevented. The result? The root hairs of seedlings didn't elongate; intracellular bacteria did not replicate within root cells, nor did they lose cell walls. Similarly, when sterile seeds were grown in a sterile medium, no superoxide was produced.

So, the superoxide breaks down the bacterial walls and its components are absorbed. The nutrients go first into the water-based gel that fills the periplasmic space and then are moved across the cell membrane into the

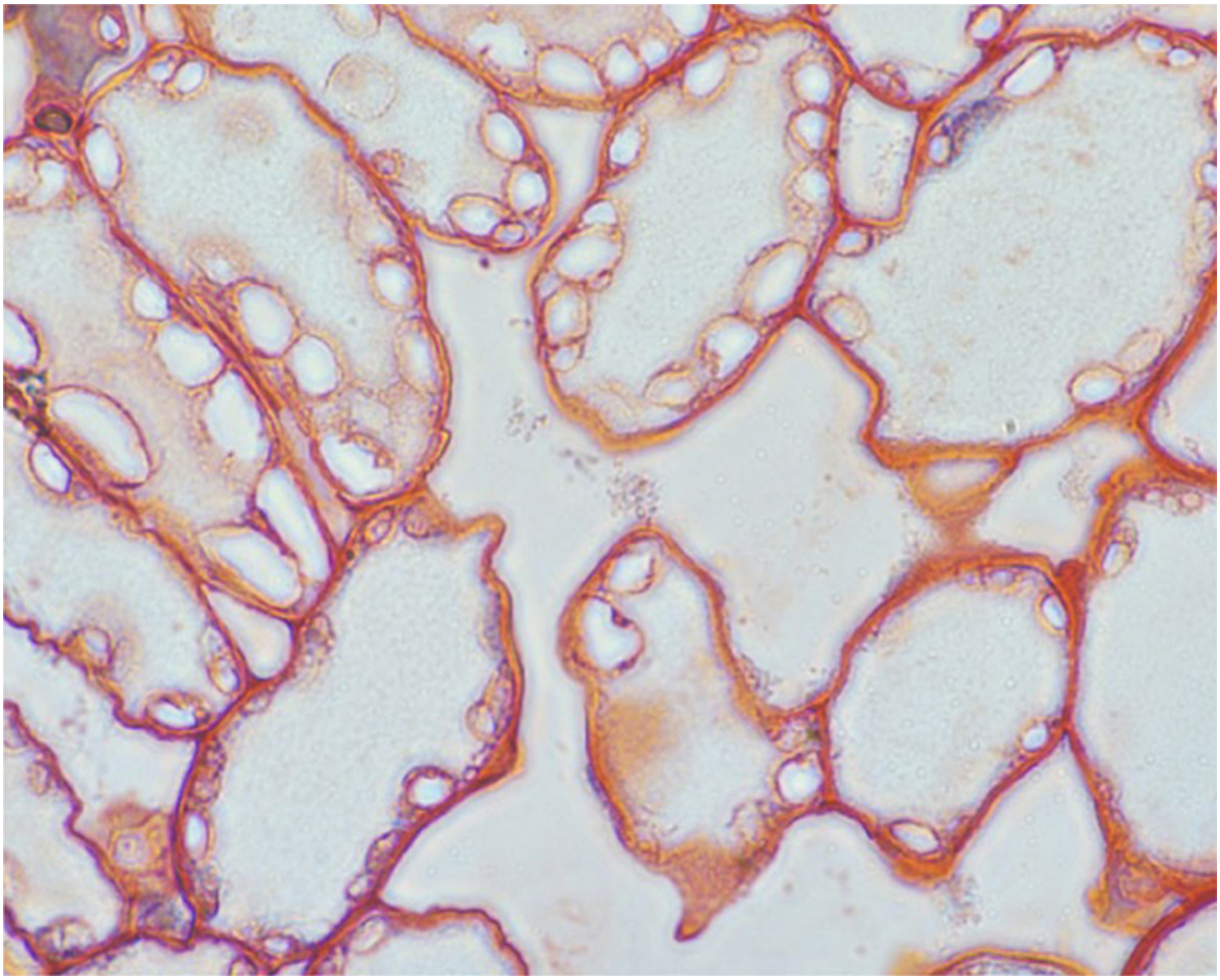
cytoplasmic space. The cytoplasm circulates around the cell at about 10 micrometers per second, and this motion (not so incidentally) mixes the ethylene throughout the periplasmic space so that elongation of the meristem cell (which is what drives the root deeper into soil) is even.

Meanwhile, the bacterial ethylene mixes with the plant ROS. The end products of this reaction are hydrogen peroxide and carbon dioxide. The CO_2 mixes with water and forms carbonic acid (H_2CO_3). Acids lower pH, which also weakens ROS, preventing damage to the plant's own cell walls.

So, the bacterial ethylene lowers the severity of ROS, but the bacteria also react to the wall removal in another way significant to the soil food web model, by again using arginine, this time producing nitric oxide (NO). (The same happens in human cells.) The nitric oxide serves as an antioxidant to superoxide, reacting with it and weakening it to stop further damage to the bacteria. It also combines with the superoxide to make peroxynitrite, which in turn is catalyzed by CO_2 (from the ethylene reaction) to form nitrate. The plant absorbs these nitrates by moving them from the periplasmic space across its membrane. Wow. Bacterial wall components plus nitrates must make a great meal.

In short, the plant and bacteria need to prevent too much activity by ROS. Of course, different kinds of bacteria show differing resistances to these ROS, just as different plants produce different strengths of ROS. This is probably why a bacterial species can take part in the rhizophagy cycle of one plant but not another. The relationships between plant and rhizophagy bacteria have evolved to match.

Some species of bacteria have cell walls that resist oxidation by ROS. They produce chemicals that protect from decay. These bacteria can overtake a plant by, for example, moving back out into the rhizosphere and consuming excessive amounts of exudates (which can cause problems with competing rhizospheric populations). They can even overwhelm the root surfaces, so no rhizophagy or absorption takes place. Again, each of the symbionts evolves until the needs of both partners are satisfied efficiently.



Intracellular endophytic bacteria, collecting in the periplasmic space between the cell wall and the cell membrane.

GO FORTH AND MULTIPLY

Enough chemistry. Without the constraints of a wall, turgor takes over and the naked bacterium (protoplast) becomes a spherical L-form. Meanwhile, the plant cell is using enzymes to assimilate the disassembled wall molecules. That periplasmic gel is full of them.

Let’s be clear. There is no difference between nutrients obtained via rhizophagy and those obtained from the soil via diffusion. Either way, liberated nutrients move across the cell membrane through the embedded protein channels, where they are available in the cytoplasm for use in biosynthesis and metabolic processes. Bacterial nitrogen is nitrogen, and bacterial phosphorus is phosphorus, be they cell wall components or part of the nutrient mix absorbed from the soil.

This is a good time to review which nutrients are in a bacterium's wall. A bacterium's wall is made of peptidoglycan, which contains sugars and amino acids. Sugars consist of carbon, hydrogen, and oxygen. Amino acids, on the other hand, can contain lots of nitrogen, iron, sodium, zinc, selenium, calcium, phosphorus, potassium, magnesium, or manganese. Note the metals: now you know why siderophores are so important.

L-form bacteria retain the ability to multiply. In fact, without walls, the process is less complicated and quicker. Much like yeast, L-form bacteria develop a bleb, a smaller bud of themselves, which normally would break off and grow. Intracellularly, however, these smaller bud-sized bacteria often form quads of much smaller cells enclosed in a structure that looks like an inflatable beach ball.

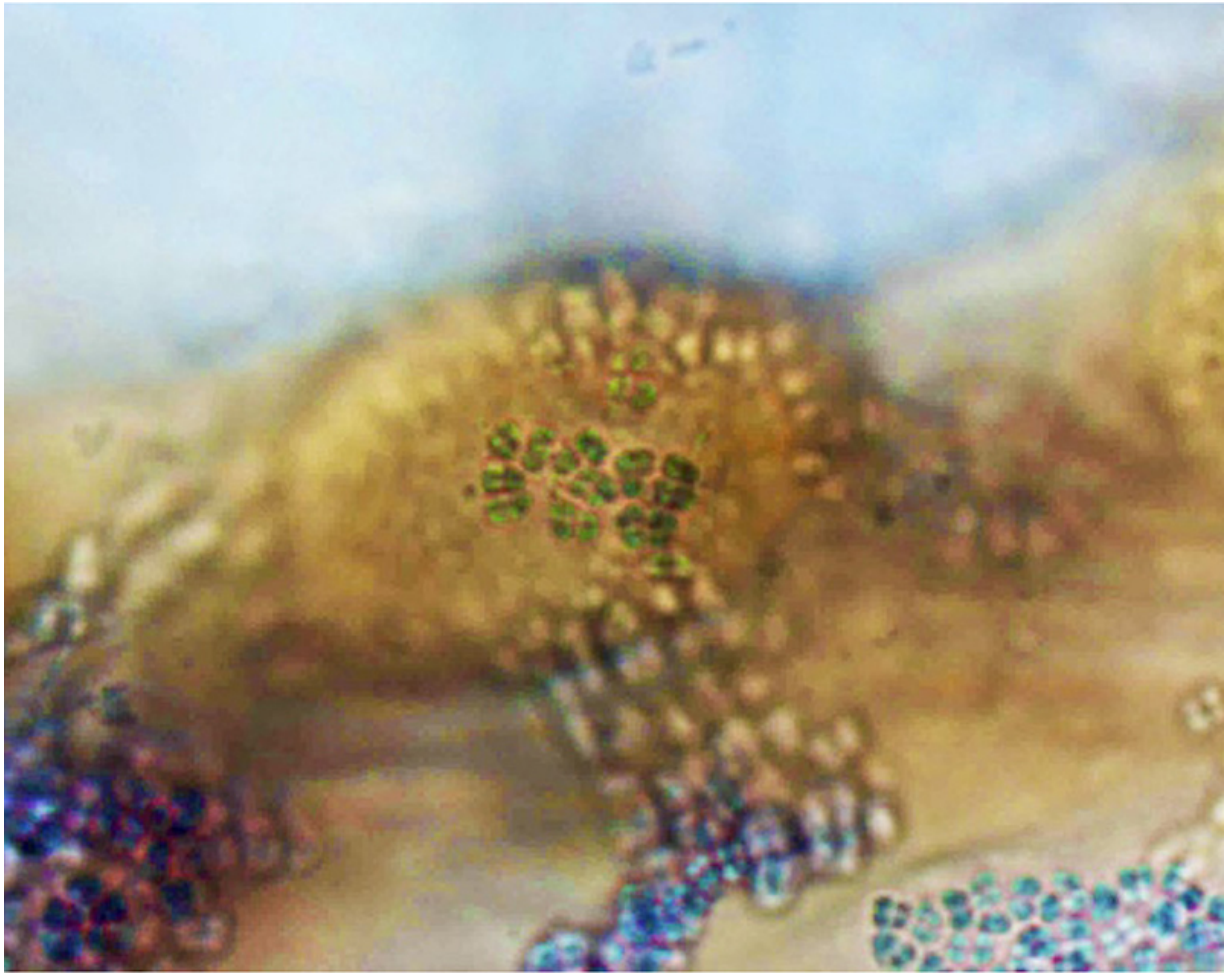
The cytoplasmic cycling moves the bacteria in the periplasm, and they rub up against the cell wall and membrane. This jostling and rubbing increases the breakup of protoplasts and keeps the ethylene produced by the bacteria moving around the cell. Picture a very thin space between the plant cell wall and the cell's membrane. (A balloon in a box works.) The pressure of turgor inside the cell pushes the membrane against the wall, but there are bacteria trapped in between the wall and the membrane.

ETHYLENE TRIGGERS ROOT HAIR GROWTH

There is only so much room in that periplasmic space, and as bacteria increase in number and circulate, at some point enough get trapped in a bulge, a trichoblast, and ethylene concentrates instead of circulates. This concentration triggers the bulge to elongate into a tubular root hair.

A couple of points. A root hair is part of an existing cell, not a new cell. This hair grows from its tip, in spurts. There are changes in the pH both inside and outside the hair tip, and ROS is produced from the membrane, where growth is occurring.

It appears ROS and changes in intracellular and extracellular pH work in conjunction to regulate hair tip growth. Only the tip grows, and this growth accelerates when the cell wall pH at the tip goes down (acidification). It stops when the cell wall pH becomes alkaline. Fluctuations in the pH may be what causes hair growth to occur in oscillations. Production and retention of ROS may be what causes the root tip to burst open when there is a high enough concentration of bacteria. This can happen many times during the growth of the hair.

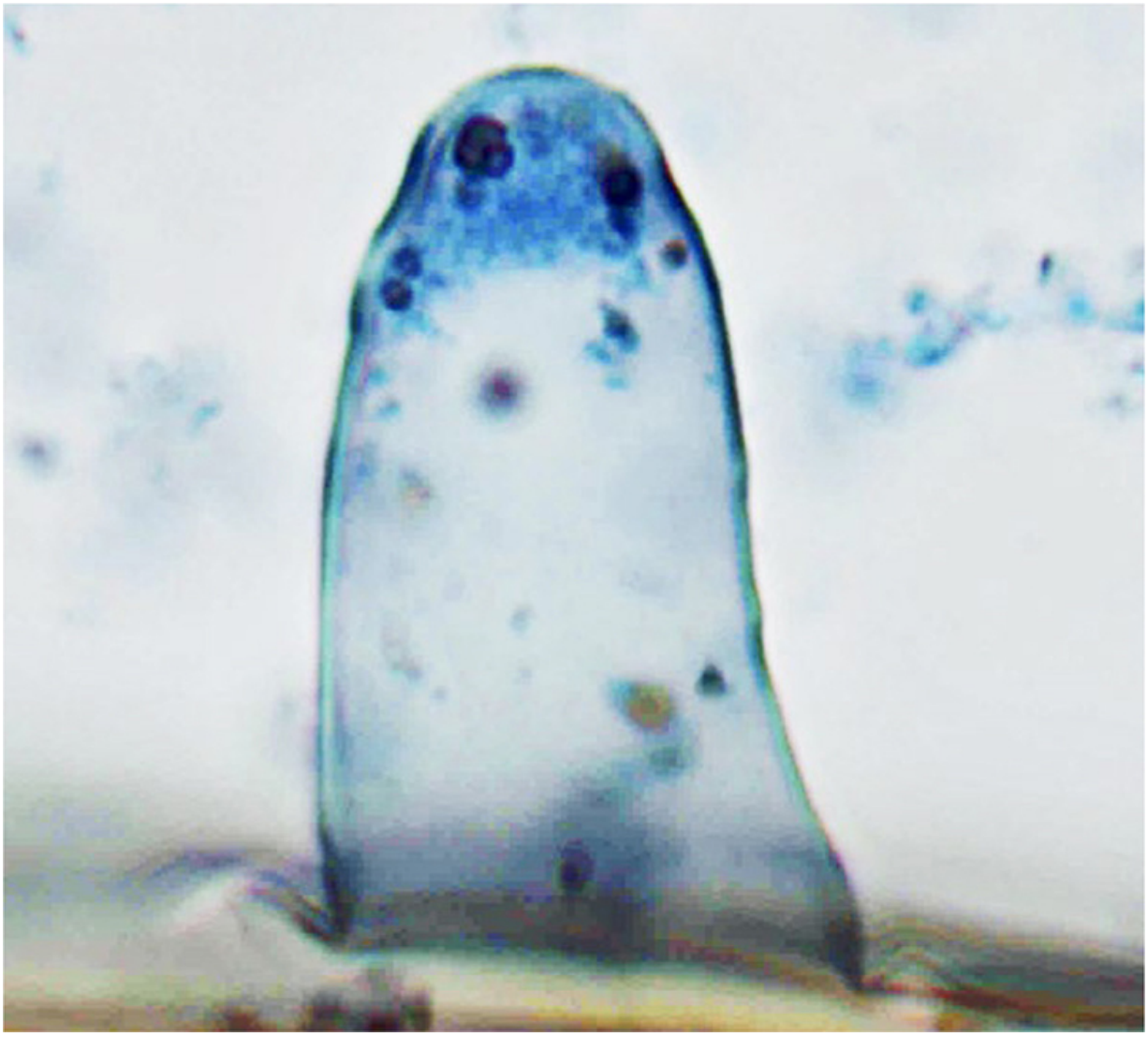


Without walls, L-form bacteria multiply, often forming quads.

Circulating bacteria continue to move into the elongating root hair; they may be trapped there by the cyclosis. And remember: the circulating endophytic bacteria also produce nitric oxide, which is converted to nitrates inside the plant; so the plant gets nutrients from bacterial cell walls as well as nitrogen produced by the bacteria. In any case, when there are enough bacteria, the whole group is ejected out into the soil along with ROS and ethylene. Then the tip closes again. More of the protoplasts in the cell gather, and more ethylene and ROS are produced—and again, there is an ejection. After several such spurts, growth stops and the hair dies.

By now meristem cells with root hairs have moved to the end of the differentiation zone. Below, new hairs in new meristem cells will continue to form—provided there is a supply of rhizosphere bacteria to become rhizophagy endophytes. The takeaway? Keeping a diverse and healthy

population of bacteria is just as important to the rhizophagy cycle as it is to the diffusion system.



The formation of a root hair is triggered by bacterially produced ethylene.

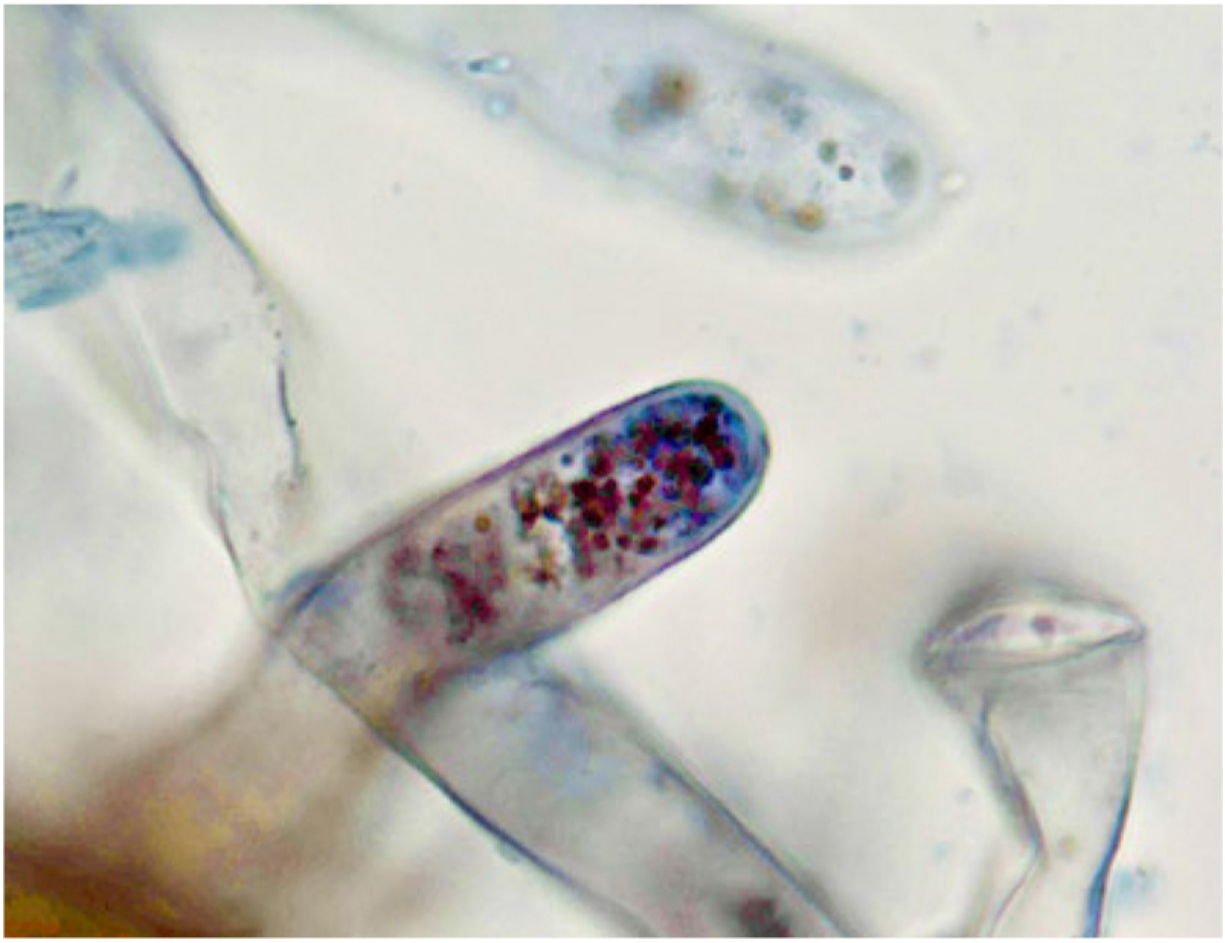
Those bacterial expulsions are caused by waves that form at the base of the growing hair and sweep upward through it. As the wave moves, the force of the cytoplasm squeezed into the hair must push the tip open—there is nowhere else for it to flow. The bacteria move out like surfers riding a curl. (Well, maybe only the ones that have done this before, the rest simply wipe out.) The pictures prove it.

Several ejections of bacteria occur over the hair’s short life, not just one. Each expulsion of bacteria is accompanied by what appears to be leakage of

superoxide (remember, ROS production continues as the hair grows). Ethylene, too, leaks out of the open tip. Is this to lower concentrations that build up because of the wall-less endophytes?

Back in the soil, again, after expulsion from the root hair, L-forms rebuild walls using soil nutrients. They live the free, rhizospheric life until that certain something—perhaps an exudate signal from the plant—calls them back to an endophytic existence.

The rhizophagy cycle repeats itself continuously as new root hairs are formed.



A great population of bacteria at the tip of a tomato root hair.

Bacteria at a tomato root hair tip, ready for release. Note the clear area, a cytoplasmic wave pushing up into the tip.

Expulsion of bacteria is visible at the root hair tip.

NOW STREAMING; COMING ATTRACTIONS

Thanks to modern microscopy we can now actually see the root hair growing during the cycle and the rhizophagy bacteria in it. The root hair swings like a pendulum as it oscillates. The first time I saw the video (pnas.org/content/suppl/2007/11/27/0708586104.DC1#M1) and then stills of the process, my own hair stood up on my head (and I don't have much). Bob's my uncle. How do scientists do it? I have already mentioned CLSM. In addition, stains are used to see some of the processes in the rhizophagy cycle.

The rhizophagy cycle is a very fertile area for research, no pun intended. Scientists are recognizing it as an important, perhaps even critical, additional way for plants to gain nutrients, and knowledge about how it works is increasing apace. Many more discoveries and lots more surprises

are yet to come. In the meantime, we must work with what we have. Just know to expect more.

BACTERIAL SYMBIONTS

You may be interested to know that the genera of Gram-negative bacteria involved in rhizophagy are Alphaproteobacteria *Bosea* and *Methylobacterium*; Betaproteobacteria *Achromobacter*; and Gammaproteobacteria *Acinetobacter*, *Klebsiella*, *Micrococcus*, *Pantoea*, and *Pseudomonas*. Gram-positive bacteria seen in rhizophagy cycles include the genera *Bacillus*, *Curtobacterium*, *Microbacterium*, and *Paenibacillus*. Someday you may see these listed in small print, on the label of a biofertilizer or another product derived from endophytic bacteria.

All rhizosphere bacteria appear to be candidates to participate in a rhizophagy cycle. It is probably up to the plant which specific bacterial strain it chooses to use. That doesn't lessen the selective nature of rhizophagy cycle participants; thus, for example, endophytic bacteria that have a beneficial effect on some grasses do not benefit dandelions or tomatoes.

MUTUAL BENEFITS

What do the bacteria get out of participating in the rhizophagy cycle? Clearly they benefit from the exudates that attract them to the root rhizoplane. The plant exudates contain carbohydrates, amino acids, vitamins, and organic acids—all necessary for bacterial metabolism, growth, and reproduction. Feeding on exudates increases bacterial populations, so much so that there can be up to ten times more bacteria in the rhizosphere than in soil elsewhere.

More specifically, the rhizophagy cycle increases certain bacterial populations. Isn't this the goal of life, to sustain the family's genetics and pass them on to a new generation? The periplasmic space in the meristem cell becomes a nursery for quickly multiplying bacteria; there, they get a better place to live, free from the competition for space and nutrients that exists in the rhizosphere.

Still, you may want to take pity on bacteria. The plant will continue to bombard them with superoxide, so they must continue to fix nitrogen for the plant, as an antidote to the ROS. At the same time, they need the

carbohydrates from plant exudates to live. If bacteria don't stimulate growth, they don't get more exudates. Are endophytic bacteria trapped into feeding the plant to survive?

And what's in it for the host plant? As more and more is revealed about this complicated system, it seems the rhizophagy cycle is operated by our plants, and we are rooting (sorry) for them. They sure know how to manipulate bacteria. Obviously, plants benefit from increased nutrients as a result of the rhizophagy cycle; these come from the increased populations of soil bacteria undergoing the diffusion process as well as intracellularly, from wall disintegration, cell leakage, and nitrogen production.

NUTRIENT UPTAKE

Which nutrients are we talking about? And more to the point, what percentage of a plant's total need for a given essential element is supplied by the rhizophagy cycle? These questions are being studied intensely. You now know enough about the composition of the bacterial cell wall to envision its amino acids being disassembled, moved into the cytoplasm, and consumed by the plant. But other things are going on. A lot of endophytic bacteria use siderophores, for example, and carry metals collected in their bodies, some of them in their own cell wall. True, bacterial walls make very small snacks, but the stockpile of them is huge.

It is difficult to determine how much of a plant's nutrient mix comes from degradation of intracellular bacteria versus absorption straight from the soil, but there have been a few studies on what, exactly, is absorbed by the plant from rhizophagy bacteria. One study of one variety of grass, for example, suggested that as much as 30% of the plant's nitrogen was coming from the rhizophagy cycle. Experiments with wheat also show modest nitrogen uptake as a result of intracellular bacteria. More study is needed and coming.

ROOT DEVELOPMENT

We now understand root hairs are not merely formed to increase a root's surface area (by about 30%) for greater absorption of nutrients and water. That third-grade notion is replaced by knowledge that endophytic bacteria have everything to do with development of root hairs and roots in general.

Adsorption of water and nutrients may simply be a side benefit of rhizophagy. (Imagine that!)

The proof's in the pudding: we witness what happens to root development when seedlings are deprived of these bacteria. What is the real purpose of those hairs—absorption or placement of rhizophagy bacteria into new soil? It is time to re-examine the function of root hairs.

Bacteria, not mineral absorption, cause root hair formation. How wild is it that roots developed from seeds sterilized of their bacteria generally don't develop root hairs and don't elongate? Return the bacteria to the seedlings, and the hairs appear. It is the same with seeds. Put the bacteria back on the very same seeds, and growth returns to normal.

Exciting research is beginning to unlock the mystery of why endophytic bacteria have such a demonstrable impact on root development. It most probably has to do with that ability to produce ethylene, the cell elongation-triggering phytohormone that both plants and bacteria are able to produce.

Bacterial ethylene reacts and combines with that specialized wall-stripping ROS, superoxide, to form hydrogen peroxide, a weaker oxidizer. In other words, the ethylene moderates the action of ROS. This makes it an antioxidant, and it is the reason rhizophagy bacteria are not destroyed, just stripped of their (delicious) walls. The population of L-forms grows, and they make root hairs, again using ethylene. (And you thought it was just used to make plastics.)

What's more, rhizophagy bacteria secrete phytohormones (auxin, cytokinins, gibberellins) other than ethylene, and these are involved in root system development too. Future investigations will reveal the full extent to which these bacteria control plant root hair growth.

IMPROVED STRESS TOLERANCE

A plant involved in the rhizophagy cycle has to protect its own cell walls from oxidative agents. These are, after all, capable of breaking down any cell wall, even the plant's own. Since reactive oxygen can damage the host too, most produce antioxidants (mostly enzymes) to prevent this. Antioxidants are also used to deal with other stresses the plant encounters.

Similarly, the incursion of intracellular bacteria has to be tolerated by the host plant. Any number of genetic adaptations developed to negotiate the rules for the symbiosis, and expression of these may not be limited to dealing with the guest. Being able to deal with ROS in root meristem cells

readies the whole plant to deal with ROS. Plants become stronger overall when stressed by a little ROS—better able, say, to tolerate cold, heat, or wind, thanks to their defensively thickened cell walls.

You get the idea. The presence of endophytic bacteria increases a host plant's tolerance of abiotic stressors. Study after study has confirmed this induced systemic tolerance, and the hunt is now on to figure out how to commercialize endophytic products for use in agriculture and horticulture.

IMPROVED DISEASE RESISTANCE

Bacteria populations returned to the soil can be useful to the plant in their non-endophytic state. For example, pseudomonads produce antifungal compounds; when some of these bacteria go back into the soil, they end up colonizing fungi for nutrients, weakening them and deterring them from attacking the plant. It is also very likely that some of the bacteria in the rhizophagy cycle are producing metabolites that upregulate a plant's defense genes, helping it to deal with pathogens.

PRETEND YOU ARE A BACTERIUM

All gardeners know there is a big difference between reading about a great garden and actually visiting it. The same applies to bacteria. It is one thing to read about them and another to see one in the flesh, at the right size, living among its fellows. For just a moment, become a rhizophagy bacterium. This will help you understand what you have learned.

Say you are rod-shaped, and there are rod-shaped bacteria all around you. (A human might take you for a colorful Cheetos.) There are other-shaped bacteria too, and archaeans, with their distinctive cell walls (but who complains when there is plenty of space and lots to eat?). Oddly, you can't just do your usual crawl. The liquid you are in is thick, and movement feels more like wading through chest-high mud than swimming. You can feel the Jell-O-like viscosity, and it is strange how you have to wave your arms in circles to move about, how flipping and twisting are suddenly effective ways to progress through your surroundings. There is a feeling of weightlessness as you tumble about, doing somersaults, flips, and corkscrew twists.

You are alone at first but are quickly surrounded by others. You stick to some. Some trade genes with you. You can act as a loner, but sometimes

your body fills with molecules that tell you to do something and you do. Your friends do as well. You light up. You move as a group. You sense your neighbors and communicate.

The protective features of your new biofilm home, constructed when you all pooled your stickiness, make it possible to move through tunnels, find food, and live without much fear of those paramecia and nematodes you keep hearing about. Unimaginably big, relatively speaking, they have a difficult time getting through the biofilm, thank goodness, so you won't be one of the 10,000 bacteria that will be lost that day to every single paramecium. Stay away from edges and anaerobic pockets and watch out for smelly acids, and things should be fine.

If you leave the colony, you might stumble into any number of neighboring colonies of different bacteria. You might find yourself attracted to metals. You might get eaten by other bacteria or protozoa or nematodes or—if you don't watch where you are going—a root. There sure are some neat things to eat in the soil, and you turn out to be one of them.

Your ancestors were brought to this patch of soil in the coat of an ancient seed. Some forebears who were on that seed went through huge tubes filled with water and sugars inside a plant; others swam through the cell wall lattice and made it into aboveground parts of the plant. Either way, they established themselves—finally winding up in a flower, which folded in on itself—and became entrapped in another seed.

Some of your ancestors disappeared in these seeds, not to be heard from other than an occasional notice that you have a distant relative in the soil of a nearby field. Meanwhile, you and your cousins grow up, living off the ample exudates that are delivered like clockwork. Food just appears. You would be suspicious if your parent hadn't signaled what was going on. (Where is Mom? She was here 20 minutes ago.) As long as you stick to the exudates, all is well. Stay away from those *E. coli* metabolites and anaerobic pockets. They might make you sick, and the latter can kill.

You learn the protocols and processes of biosynthesis. You find materials, hammer out a few with bonds, and use them to build whatever you need. Some you use to regulate your metabolic functions. You might even saddle up to a neighbor, put out a tube, and trade little bits of DNA. Fun. You never know what you will get and how you can use it to better your situation.

Ah, to grow. *This* is how you make your contribution to the colony. You grow, and that leads to duplication. A sustainable population that can pass on traits is the goal of bacterial life (the peaceful ones), and you are meeting it. Over and over, every 20 minutes, this is made clear. You realize that somewhere along the way you picked up a trait that is now shared by others. You created a strain. Way to go!

Then one day while minding your own business, moving lazily through the biofilm of your colony, hungrier than normal due to too much competition for food, you detect the delicious smell of buttery popcorn. (I know the lure of fresh butter: Hotel Bar Butter, a New York favorite, was sold by generations of my family.) You have only unbuttered popcorn, so you attach yourself to the wall of the plant root cell from which the tantalizing odor is originating.

You are leaning right up against the root, drooling enzymes, when the wall develops breaks and that smell intensifies! You drool more enzymes and follow the trail, swimming through the cracks in the wall and finally finding yourself inside a plant cell. You are exhaling ethylene, and you note this place is different from soil, a gel-like liquid with a bunch of your siblings moving around against the inner wall in a big circle. The current is strong, and you are swept into it as it circulates around the cell.

Some instinctive urge to repay the cell for the great circular ride (or perhaps to lighten your load, so the ride is more fun) causes you to convert one of the amino acids you picked up out in the soil, from the plant's exudates, into more ethylene. It is a gas, and you can't hold it in, so you release it into the eddies. The ethylene coats the cell wall.

The plant that lured you in now treats you like you are in a car wash. It sprays a mist from nozzles in its membrane, and you notice your cell wall disappearing. It doesn't hurt; you can still fully function, but now you leak from your exposed, embedded membrane proteins. It would be embarrassing if the same thing wasn't happening to your friends.

You fix nitrogen into nitric oxide and combine it with superoxide to make nitrates to stop the wall disintegration. This it does, but the plant takes the nitrates and uses them to grow.

Where does the wall go? What happens to leaked electrolytes? Why are you feeling like you have been to a plastic surgeon and had your membrane tightened? You look at your siblings forming smaller forms and quads, and

instead of the familiar rod shape, they are now all round bubbles. You realize your own body has become positively spherical.

You are being cycled around, completely naked, inside the plant root cell wall. Everything still works, though, so you enjoy the ride.You bump into a plasma membrane, but it only pushes you back into the current. Still, you can see holes where the components of your wall are disappearing.

You now throw all inhibitions to the protoplasmic forces and multiply right there in front of the others. No wall, so you can easily and quickly form a bleb—and another, and another. The next thing you know there are four of you in a bubble, like four adult humans in a Volkswagen bug. When it gets too crowded, you line the others up and use ethylene to soften and then push a portion of the plant cell wall outward, forming a tube, to make more room.

Then, the most amazing thing happens—something that makes the whole two-day journey worth repeating: a tidal wave blasts into the tube that you helped form and pushes you out through the tip. When you catch your aerobic breath, you find yourself and your close friends back in soil. What a short strange trip it's been! Was that only two days? How many new clones are out there in the soil with you? Power is in numbers.

Famished, you find nutrients in this fresh soil to rebuild your wall. At the same time you fill your pantries with arginine and other components from the plant's exudates. You divide and divide again. Life returns to normal.

Ah, but there is that butter smell again and you remember the great wave ride. Popcorn and butter! The spinning around, all that budding! Fixing nitrogen. The heck with just eating exudates, you just have to return to the inside of a new cell and experience the great ride over again. And this time you will take that curl standing up. And so you do.

PRETEND YOU ARE A PLANT

Your feet, er, roots are in rich, well-aerated soil. There is a wonderfully warm film of water around each soil particle and your roots. You are surrounded by 50 or so different kinds of bacteria, fungi, nematodes, and protozoa that your ancestors coded you to recognized and, in some instances, domesticate. This is a great place to live. All you have to do is develop new roots, get nutrients to grow a few leaves, and the next thing you know, you are photosynthesizing away.

You make carbon from the sun to biosynthesize organic compounds to serve as soil exudates. You cook up some great dishes, which you leave in the soil to attract bacteria and fungi. You combine lots of nutrients with your carbon, and once microbes discover a cache like this, they call their friends. An orgy of molecular decay ensues. All manner of essential nutrients are flowing toward your roots.

You are patient. For a few days you can live off the microbes and starch that were cached in the seed from which you sprouted. Those leaves really help jump start things, however. The next thing you know, the nutrients in the soil are converted by all those microbes you attracted into a form you can eat. This is too good to be true. The microbes think you are foolish to produce exudates for them, but they don't know you are setting up a system to take advantage of them. The bacteria and fungi attract nematodes and protozoa; they in turn eat some of your bacteria and fungi. It happens every time.

The waste products that result from all this activity are in the form of nutrient ions, just the kind of food you like and need. You use your knowledge of chemistry to diffuse them into your root cells, where they are used or transported to other places in you.

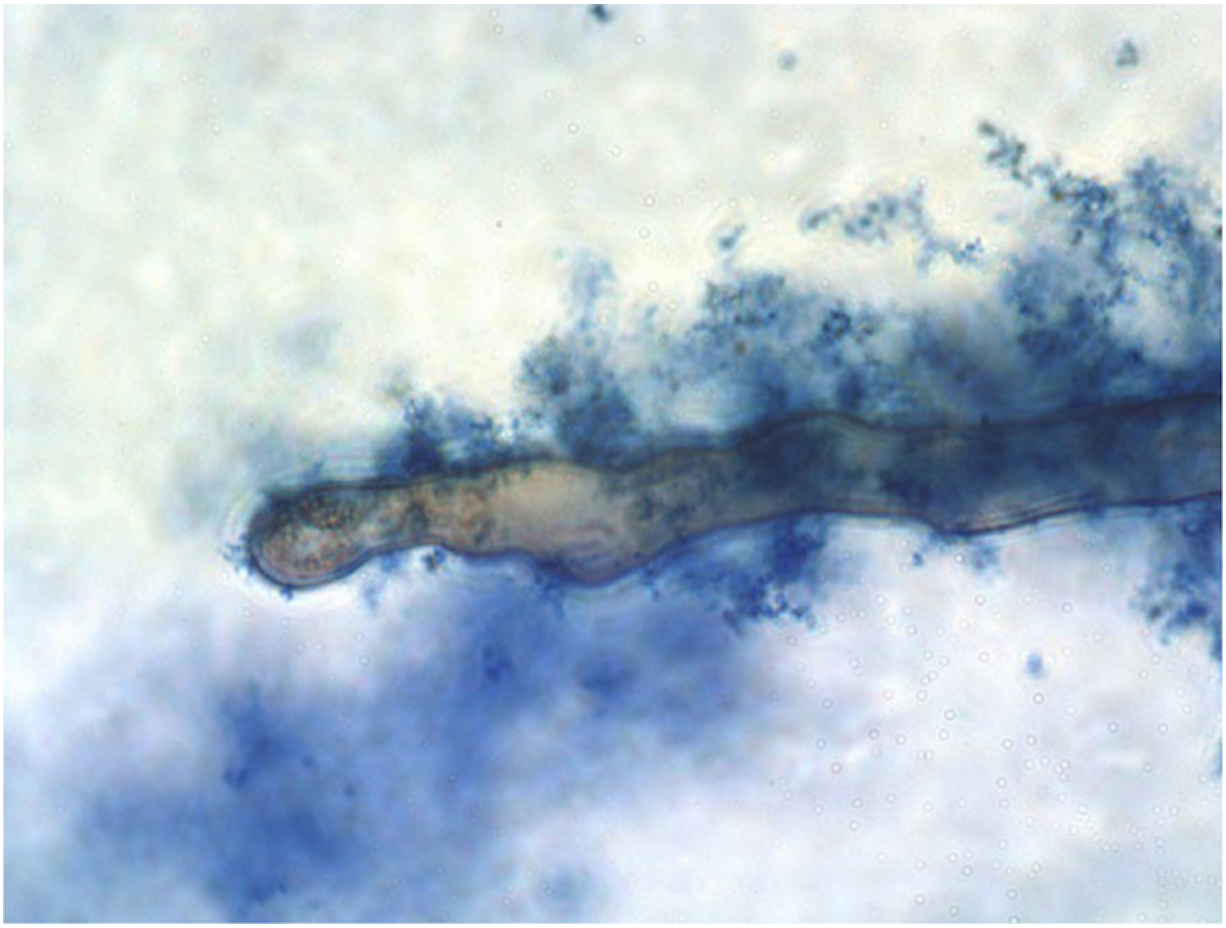
This is great food, but you are still hungry. You need more. Fortunately, you remember you are a plant, and you are in control. You remember a plant friend, Seymour, who could never get enough because he was not kept organically. Always screaming "Feed me!" Let that be a lesson: no one else is going to help you—especially not humans who try to foist that artificial junk on you.

You concoct a special exudate and attract special fungi. In return for the special mix and a place to live, in between root cells, these go out and get you water and additional nutrients. They always bring pets, too—bacteria all along their hyphae that help solubilize phosphates, which was hard to get on your own but is suddenly plentiful.

You discover you have a legume plant friend who can attract bacteria that fix nitrogen. You try and duplicate the act, but the nitrogen you get is mere spillage from her nodes. (Later, as some of her leaves decay on the ground, you might get some nitrogen newly put into the soil.) You are still hungry, and you need more.

Then you realize you are not using your exudates efficiently. They are growing a great population of microbes in the soil, but how do you get at

their nutrients more directly? Why support nematodes and protozoa? Experience (and plant business school) taught you to cut out the middlemen. And, wonders of Nature, you find a group of bacteria in the mix willing to work with you. These agree to become temporary endophytes. All it takes is popcorn and a bit of butter. Once you get them inside, you entertain them and make sure they want to keep coming back.



There is usually more than one expulsion as the bacteria multiply.

As they eat their buttered popcorn, the bacteria open the wall of one of your new cells. Once these bacteria enter your roots, they let you gently shear off their walls, freeing them of that weight and constraint. The constituent parts of these bacterial walls are moved from your periplasmic space to inside your protective membrane. You are no longer hungry. And dessert: you get to enjoy leaking nutrients from the now wall-less bacterial forms.

Realizing you want them to come back again and again, you push them onto the best amusement park ride ever conceived (sorry, Mr. Disney)—the Cyclosis Coaster and Hairy Catapult. Bacteria get to ride the Cyclosis Coaster, and they get to bud in a nice safe place where they don't have to worry about parents, predators, or competing relatives. You give them some sugars from your own supply, and they seem trapped by this. Plus, they must realize that if they stopped feeding you nitrogen, they would die: their nitrogen fixation stops their own wall deterioration.

The euphoria caused by strength in numbers takes over. The bacteria are having a great time. The bonus is your promise to put them back into the soil in a location where they can rebuild walls and live a better life. You let them help you build a long tubular wave pool, and they immediately gather for the fun. And fun it is. Welcome to Hawaiian surfing conditions, right here inside your root meristem cells.

To distinguish these bacteria from bad bacteria, you learn to recognize the signals they send. You might not trust bacteria, but you have evolved to tolerate the ones that can feed you, especially when that food is nitrates. Tolerance makes you strong in lots of ways. It is like working out and building muscle. That spray you put on the entering walled bacteria requires that you toughen up too, to avoid collateral damage, as you can't help but get some on your own walls.

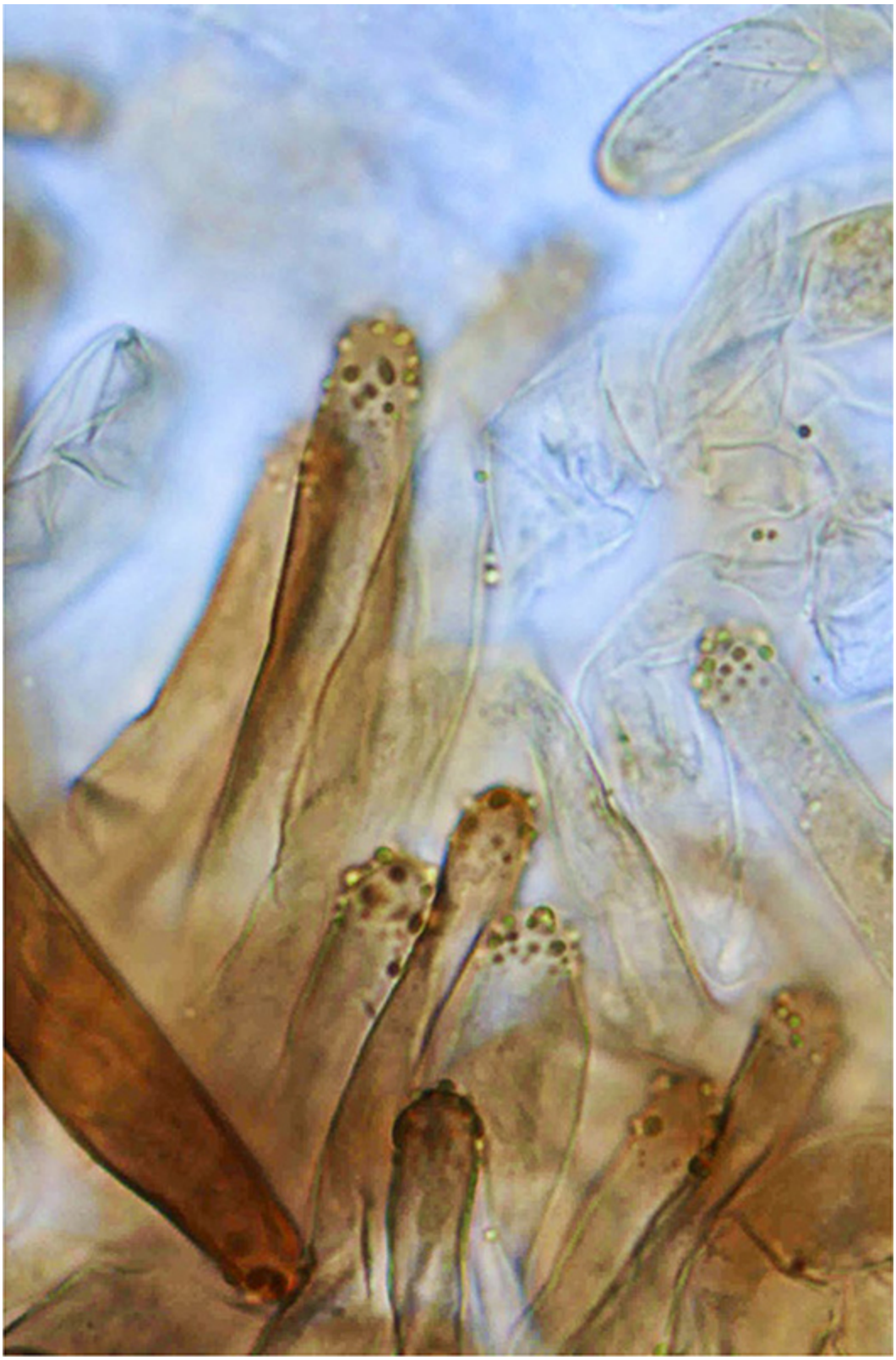
Hey! You become a better plant because you are amped up by the presence of these bacteria. All your root hairs are full of them. You experiment and discover other bacteria like different parts of your body. It isn't so bad having them there, either. You let more bacteria in where branches form on your roots. Suddenly it isn't so cold, and you find you can handle a change in pH or whatever amount of water is available. Salt? No longer a problem (up to a point). Life becomes a bit more carefree, and you feel so much stronger. Oh and the food from rhizophagy! Tastes fantastic. Everyone wins.

It is a wonderful thing to be able to build populations of bacteria internally as well as in the soil. They both feed you. They both protect you. You recognize that some bacteria really are your best friends, not enemies at all. From now on, you promise to give them more respect and treat them better—or get your humans to do so by at least trying to visualize them from time to time.

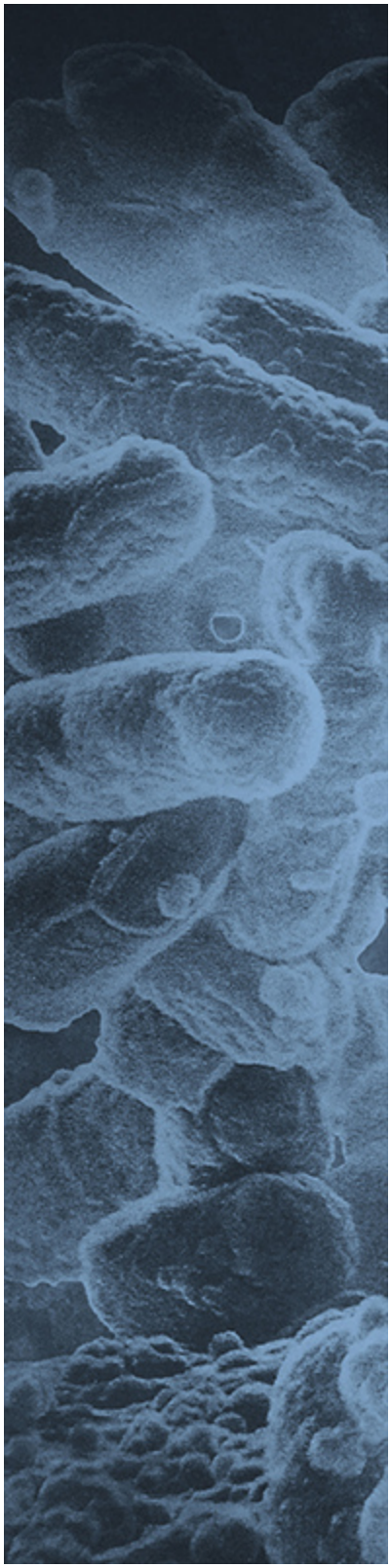
A LOT OF WORK TO DO

Research on the rhizophagy cycle is just beginning. We can take in what researchers have discovered thus far, but the very newness of the concept heavily hints that our understanding of it is only at the pioneering stage. There is much more to come and a lot more to learn. But that doesn't mean that it isn't time to educate ourselves and others about what is known to date. Armed with knowledge about endophytic bacteria, particularly those that engage in the rhizophagy cycle, we and our fellow gardeners, farmers, and growers of all kinds can adjust our cultivation practices, now, so as not to interfere with—and better yet, to assist—these tremendously beneficial bacterial helpers.

The rhizophagy cycle is new. It is revolutionary. It is happening now. It is time to acknowledge it. It's the start of a new chapter, not the end of this one.



All root hairs contain endophytic bacteria.



Growing with Endophytic Bacteria

MOST COMMERCIAL FERTILIZERS inflict incalculable damage on the soil food web and the environment. They increase climate change pressure and too often negatively impact worker and consumer health. The continued application of synthetic chemicals is wasteful and unsustainable. By turning instead to endophytic bacteria to promote growth, control pathogens, and increase a plant's tolerance to stresses, we can save diminishing resources and take a step toward reversing global warming while still feeding the world's growing population.

Endophytic bacteria are plentiful, easy to apply, and work for free—and researchers are working furiously to realize their potential for use in agriculture. The need is great. The market is huge. Identification and production of agricultural biocontrol and biofertilizers is becoming a big industry. There are challenges—manufacturing consistency and the ability to ensure plant and bacterial strain match—but science will find a way, and growers will be able to purchase and use more endophytes with greater confidence.

New products hit the market almost weekly; until now, they have been directed toward large agricultural enterprises, but they are finally starting to filter down from professional growers to home gardeners. Biofertilizers harness nitrogen-fixing endophytes. Some endophytic formulations solubilize phosphates; others just help plants make better use of fertilizers so less is needed; one product increases a plant's ability to withstand freezing temperatures. There are even endophytic fungicides.

THE MYCORRHIZAL FUNGI MODEL

Although they are not bacteria, mycorrhizal fungi are endophytic and increasingly in commercial use; they are therefore a good indicator of where things are headed with endophytic bacteria. Of course, bacteria and fungi can work together. And no question—the number of endophytic bacterial products will expand, just as the number of mycorrhizal formulations has.

When *Teaming with Fungi* came out, mycorrhizal fungi were just reaching the consumer market; now they are available at all good nurseries and grow shops. (I even advise readers of my weekly column that if a nursery does not sell mycorrhizal fungi, they should go somewhere else, as it is not up to date.) Endophytic bacteria are easier to produce in a lab than mycorrhizal fungi, but they share the problem of host—microbe specificity. A great deal of research is going on to match the right endophytic bacteria with the proper crop plants. The results will ensure many more endophytic bacteria are used both in agriculture and at home.

THE RHIZOBIA MODEL

As early as the 1800s, farmers knew soil taken from the previous year's legume beds resulted in a significantly better legume harvest the following year (though they had no idea why). Ultimately, this practice resulted in a patent for Nitragin, the first nitrogen-fixing inoculant for legumes.

Today, what gardener worth her salts doesn't roll pea and sweet pea seeds or roots in a rhizobia mix? Rhizobia are readily available even at the consumer level. Since nodule-forming rhizobia are limited to use with legume crops, scientists are experimentally inducing other plants to host non-nodulating types to fix nitrogen endophytically. There is a great deal of promise here, particularly with corn and wheat.

Several genera of bacteria are capable of fixing nitrogen, including *Azorhizobium*, *Bradyrhizobium*, *Rhizobium*, and *Sinorhizobium*—collectively, rhizobia. The industry has expanded to meet different agricultural needs by adapting to the different ways plants can be inoculated. Thus early rhizobia inoculants were peat-based. Now you can buy these endophytes in vermiculite and in liquid and specialty bases, such as clay. This is happening all over the world.

Azospirillum

The genus *Azospirillum* is one of several Gram-negative PGPBs that can promote plant growth by fixing nitrogen under low oxygen conditions. There are 15 *Azospirillum* species and a few dozen inoculants. The first species, *A. formosense*, was discovered in Taiwanese soil; a Brazilian species, *A. brasilense*, naturally found in the roots of grasses there, is now in heavy agricultural use around the world. Different strains have since been identified in Russia, Canada, Korea, Japan, Australia, and Pakistan; they

have been used in commercial agricultural since 2009 and, as of this writing, the industry has employed over 3.5 million doses of these early endophytic bacterial products.

Using *Azospirillum* inoculants can result in a whopping 25% reduction in the use of commercial nitrogen fertilizer. (Wow.) And *Azospirillum* products can be used, as one label notes, on "all agricultural and horticultural crops," including rice and wheat.

While they were initially commercialized for their nitrogen-fixing abilities, it has since been demonstrated that these endophytic bacteria produce auxin, ethylene (and its precursor ICC), cytokinins, gibberellins, and abscisic and salicylic acids. *Azospirillum* bacteria can also produce siderophores in low iron conditions. Some *Azospirillum* strains solubilize phosphates. Some control pathogenic fungi, both through production of metabolites, and by cannibalizing them and delivering them to the plant host as nutrients. They are useful, indeed. It seems *Azospirillum* does it all.

Azospirillum products are used on several different kinds of plants, but it is monocotyledons (grass and grain crops) that benefit the most from these bacteria. Although they appear naturally in the rhizospheres of sugarcane and C_3 grasses in South America, they are absent in many places around the world, and applying them to commercial crops of corn, wheat, and barley is gaining in popularity internationally. Grape growers are experimenting with *Azospirillum* products and having success, and many who cultivate cannabis are starting to use these products to feed their plants and prevent transplant shock. This is a sure indication of their effectiveness, as not only are cannabis growers usually ahead of the game, they are also great researchers, so expect more information on using *Azospirillum*.

Azospirillum is applied in planting furrows, on seeds, or as foliar sprays. Its commercial use is clearly pointing the way to the future, proving the worth of endophytic bacteria.

Bacillus

Bacillus amyloliquefaciens is another PGPB, and like *Azospirillum*, it does the heavy lifting in more than one area. In addition to nitrogen fixation, it solubilizes phosphorus, potassium, and zinc. If that isn't enough, *B. amyloliquefaciens* also produces phytohormones, siderophores, and an enzyme that takes out fungi. No wonder, then, that this endophyte has captured the attention of growers.

Bacillus subtilis can be endophytic and is easy to store for long periods of time, which is very important to commercialization. This rod-shaped bacterium readily turns itself into an endospore (during times of great stress, all *Bacillus* spp. can do this). As an endospore, its genetic material is surrounded by a protective coat, which is tough enough to have enabled *B. subtilis* to survive six years in outer space. Obviously, it can survive radiation as well as heat, cold, and lack of food.

In progress: an experiment to determine the effect of different *Azospirillum* strains on wheat growth.

Bacillus subtilis is very competitive and can outcompete harmful fungi both in soil and on plant surfaces. It produces chemicals that take out its competitors and slows down their population growth. *Bacillus subtilis* can even prevent spore formation by its enemies, thus protecting nearby plants from competition and pathogens; it also helps solubilize phosphates. So it isn't surprising that various products based on strains of *B. subtilis* are in the market. They are used on leaf vegetables, potatoes, strawberry plants, grape vines, all kinds of pome fruit trees, nut trees, and cannabis. They are used to control powdery mildews (pathogen: *Botrytis* spp.), bacterial soft rot (pathogen: *Pectobacterium carotovorum*), and citrus canker and other

bacterial spots and blights of leaves, stems, and fruits (pathogen: *Xanthomonas* spp.). Gardeners should clearly look into *Bacillus subtilis*, which is available via the web and at knowledgeable nurseries and grow stores. Soon all nurseries will sell it.

Another species, *Bacillus thuringiensis*, is a biopesticide already used by many gardeners and farmers. It is an endophytic bacterium that causes caterpillar death. The bacteria carry spores, which produce crystals that are poisonous to larvae of the order Lepidoptera (butterflies and moths). *Bacillus thuringiensis* subsp. *kyushuensis* and *B. t.* subsp. *israelensis* kill mosquitoes. These days it is easy to find these two strains, as they are used to keep water features clear of mosquito larvae; they are sold at virtually every nursery.

Back to *Bacillus thuringiensis*, so popular it goes by the acronym Bt. It is used so much that many are concerned about organisms developing resistance to its ways. Never underestimate evolution, as all who understand endophytes know.

METHYLOBACTERIA

Methylobacteria—aka pink-pigmented facultative methylotrophs (PPFMS)—should be famous. Why? *Methylobacterium* spp. are model endophytic bacteria for study; they have had a long association with all kinds of plants, and they are found in all organic soils after plants die. If they need to, methylobacteria can derive energy from methanol and related compounds. And they are, indeed, pink. The key thing for growers is that methylobacteria produce several phytohormones, specifically auxin, cytokinins, and gibberellins. They can also inhibit production of ethylene.

One kind of PPFM resides in the root nodules of some legumes; these endophytic bacteria also occur in leaves, where they produce UVA-protecting metabolites, shielding leaves from sunburn. But above all, methylobacteria are found in all seeds, just under the seed coat; they support the seed during germination and then stay with the plant as it grows. If removed, the result is a lower germination rate and abnormal growth. Return the methylobacteria, and germination rates go back up and plants grow normally. And get this: the germination rate of even old seeds is increased considerably by the use of bacteria in the genus *Methylobacterium*. This is an eye-opener for anyone having trouble with seeds that do not reliably germinate.

Methylobacteria are being tested on all sorts of crops. They can increase yields under abiotic stress and demonstrate antifungal and antibacterial properties. Products employing them are not as easily stored as *Bacillus* endospores, but methylobacteria are more easily stored than most other bacterial endophytes because these endophytes are capable of regeneration after freeze-drying. This is not as good a storage solution as simply being an endospore, but it works on methylobacteria. The result is that a few different strains of methylobacteria are on the market (for those who can afford them).

Helicopter spraying *Bacillus thuringiensis* subsp. *kurstaki* (Btk) for gypsy moth eradication, Lane County, Oregon.

PPFMs aid plant growth and health by their production of phytohormones. They increase a plant's content of vitamin B12, an important nutrient we seek from leafy vegetables. And some methylobacteria form nodules in host plants and then fix nitrogen. Finally, they help get rid of methanol, a waste product of cell division.

IN THE PIPELINE

While the everyday gardener has been applying just Bt, scientists have been hard at work, and other *Bacillus* spp. are now included in a growing number of commercial formulations. Look for *B. azotoformans*, *B. coagulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, and *B. pumilus* where you buy plants and growing products. Always read labels. Ease of storability, coupled with the backyard gardener's increasing understanding of how they work, will lead to even greater use and further development of *Bacillus* strains.

Lots of other endophytic bacteria, such as *Azotobacter chroococcum* and *Paenibacillus durus*, are available in mixtures with other microbes. The market is there. The task is to educate growers of all stripes about endophytes—what they are and what they can do for growing plants. After all, if all plants have them, there must be something to them.

Modern practices are ruining soils and forests, and it is becoming increasingly difficult to feed the world's growing population. In addition to costs and efficiency, much attention is being paid to sustainability, climate problems, and human health. Why, then, aren't there more products that tap the benefits endophytes impart? Because it isn't easy getting them to market. There are lots of roadblocks to commercializing bacterial endophytes. These must be dealt with before we'll see everyday use of endophytic bacteria.

One basic barrier is the problem of isolating and identifying endophytic bacteria and learning what plants they will impact. There is a great deal of diversity in individual species and strains, both genotypic (traits based on genes) and phenotypic (traits based on nongenetic factors). If you think you have a useful endophyte, you must ensure that yours can be isolated into a single strain.

ISOLATING ENDOPHYTIC BACTERIA

How do you isolate endophytic bacteria from a plant? There are several ways, most involving washing the plant (or seeds) and sterilizing surfaces. In the simplest method, the plant is then divided into tissue groups—roots, stems, leaves, flowers. These plant parts are cut into smaller pieces and further sterilized by soaking in distilled water and rinsing in ethanol and then bleach. There is more washing with distilled water, followed by shaking and grinding; the resultant powder suspension is grown on agar.

And that is the easy part. Next, the bacteria need to grow and colonies of different ones isolated until there is a single strain. Remember, we are dealing with microbes; they require the right nutrient(s) and are not always supportable in a petri dish. For one thing, most soil bacteria won't grow outside of the soil. It is also possible that the endophyte you seek has been taken out by a competitor.

Once isolated, bacteria are put through a battery of standard tests to identify potential beneficial characteristics. (Heavy on "potential": with endophytes, what works in the lab does not always prove true in the real world.) These tests include Gram-staining, cellulase and pectinate activity tests (to show the extent of wall-attacking enzymes), and a whole raft of other tests to determine auxin production, motility, and antibiotic resistance.

Perhaps the easiest part of the process (at least for those with the right resources) is identification of the isolated bacterial species and strain. Scientists extract DNA and use metagenomics to sequence genetic material, which allows an exact identification of each individual bacterium, even if from a mixture. It is all done by computer, including a comparison to worldwide databases.

POST-ISOLATION HURDLES

Once a strain is isolated and identified, it's time to link it to desirable traits in a host plant. One recent study showed 23 different isolates on seeds of rice cutgrass (*Leersia oryzoides*). All had to be tested; only ten promoted growth. This points out why each isolate needs a study of its own. This is time-consuming work.

After all the greenhouse trials are completed, you need to start field tests. Once you've come up with a viable candidate endophyte and know which plants it works with, then you have to figure out packaging, storage, distribution, and, most important, how to educate the masses. For those entrepreneurs among you, it might just make sense to hire a company, or get a university to do the job for you.

Even after you isolate and develop a good endophyte for commercial application, additional struggles are around the corner. One is beyond your control: populations of competing microbes, which in the case of endophytes can be a real problem. It may be that indigenous microbes already fill the required host niche, and they will fight to remain. (The plant may even join in the fracas.) There really isn't anything you can do except

test any product locally, on your particular plants, to see if it works as intended.

No one said it was a quick or easy process. This is just one reason why some endophytic bacterial products are too expensive for the home garden market and why some farmers and growers have not switched to using them. Costs are coming down, however, as knowledge of how to isolate, manufacture, package, store, and apply is perfected.

COMMERCIAL MICROBIAL INOCULANTS

So, commercial endophytic formulations are already available—myriad rhizobia products as well as those that contain *Azospirillum*, *Bacillus*, and other PGPBs. Many are produced by small to medium-sized independent companies. Without endorsing any, and with apologies to those not mentioned, let's take a look at what's now available.

Sure, you're smiling now. Cutting plants into ever smaller pieces is the simple part of isolating endophytes.

Bacillus subtilis is the backbone bacterium in many formulations, including Bayer's Serenade products and its Kodiak brand. Cease by

BioWorks and Vault by BASF also contain *Bacillus*.

Paenibacillus polymyxa, a nitrogen-fixing *Bacillus* relative, is used in agriculture and horticulture. *Paenibacillus* sp. strain JDR-2 is being studied: it is full of chemical agents that will be useful for bioagricultural applications. These are related to *P. dendritiformis*, *P. vortex*, and other pattern-forming endophytic strains.

Pseudomonas fluorescens, available worldwide, is beneficial when used on (really, in) chilies, annual and perennial cut flowers, cucumbers, eggplant, potatoes, and tomatoes. This bacterium moves through the vascular system of the plant and into all parts of it, fighting all manner of diseases, both fungal and bacterial.

Rhizobium radiobacter is commercially available, at least in Japan; it is used to control crown gall in roses. NOGALL, a similar product from BASF, contains *R. rhizogenes*.

Lots of other PGPB strains are used in market products, and many are endophytic: *Azospirillum brasilense*, *A. lipoferum*, *Delftia acidovorans*, *Paenibacillus macerans*, *Pantoea agglomerans*, *Serratia entomophila*, and various rhizobia.

Though it is a soil-based product and billed as a microstimulant, Mammoth P is a good example of a product that contains endophytic bacteria. Its four PGPBs—*Citrobacter freundii*, *Comamonas testosteroni*, *Enterobacter cloacae*, and *Pseudomonas putida*—are very competitive and successful, and they probably engage in the rhizophagy cycle, ensuring enough phosphorus for plants. Mammoth P jumped from agriculture use to home gardener and grower use because the company took the time to educate gardeners. More and more small growers and home gardeners can now find it wherever they purchase supplies.

Some products don’t make it. BioCoat, consisting of *Pseudomonas fluorescens*, was sold for suppression of fusarium wilt in radish, but its shelf life was too short and its disease suppression was low, so it is no longer available. One rumored dandelion product unfortunately killed lettuces as well as the weed. Nothing is lost, however, as the reasons for failure can help ensure the next success. New formulations are being researched.

KEY STUDIES

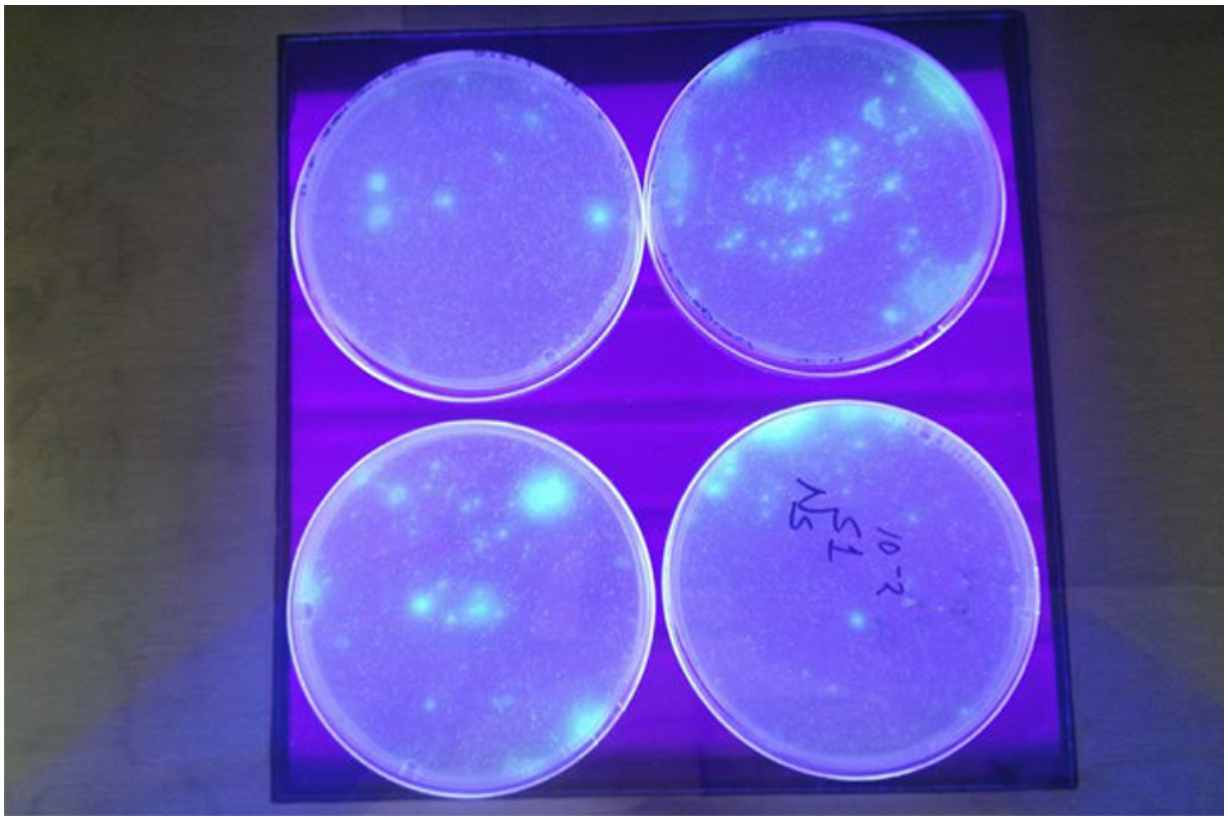
It is interesting to see the range of studies of endophytic bacteria and their results. (And believe me, I have read lots and lots of them for this book.) It

makes me want to learn the staining and isolation techniques and the microscopy to be able to join in. (Ah, to be a youngster just starting out and in need of a subject for a master's thesis or dissertation.)

What follows are just some summary examples of the good things that happen when endophytes interact with plants or at least which endophytes hold the most potential for a particular plant group. You still don't need to get hung up by the names of these bacteria. Just let them flow.

FRUITS AND VEGETABLES

Fruits and vegetables make up a large segment of the organic market. Growers looking to meet the increasing demand for organic produce have a real need to explore endophytes as biofertilizers and biopesticides. The needs of these growers are the closest to those of home gardeners, and the shipping and storage issues they exclusively face might best be addressed by the addition of an appropriate endophyte or two. Is there a way an endophyte can modulate ethylene in aboveground fruits, as happens when bacterial ethylene mixes with superoxide during the rhizophagy cycle? Endophytes that produce ACC (an enzyme that reduces ethylene formation) in roots could have applications in fruits.



Pseudomonas fluorescens really does glow under UV light.

Asian greens. Actinobacteria, Bacteroidetes, and Proteobacteria were the main phyla represented in a study of soil, compost samples, and Asian greens (*Amaranthus* spp., *Brassica oleracea* var. *alboglabra*, *B. rapa* var. *parachinensis*). More than 290 bacterial species were identified. Now this data is machine-accessible in a metadata file. The researchers hope their dataset, and the comparative studies that will result from it, will advance our knowledge of healthy vegetable crop phytobiomes and how they may be manipulated to enhance crop productivity. This is the wave of the future for endophytic research.

bananas (*Musa*). Bananas are in big trouble. Some specialists predict these favorite fruits will become unavailable in the near future, and the excessive use of chemical pesticides is a major cause. So, there is a lot of endophyte study going on here. The opportunity to help save them is a real opportunity being explored by many research projects. These have already shown that particular endophytes (among them, *Azospirillum*, *Bacillus*, *Enterobacter*, *Herbaspirillum*, *Pseudomonas*, *Rhizobium*, and *Serratia* spp.) can improve

soil fertility, which in turn supports the health of bananas. These same endophytes work as biocontrol agents against many of the pathogens that infect bananas, including *Xanthomonas*.

basil (*Ocimum basilicum*). Experiments with sterile basil seed show that seedlings are taken out quickly by fungi. When bacteria are returned, they help the seedling by establishing colonies on the fungal hyphae. This is not good for keeping the hydrophobic fungal coatings intact; they leak nutrients, which are used by the plant, while the fungi, in turn, become weak and nonlethal.

coffee (*Coffea*). Coffees host bacterial endophytes. *Gluconacetobacter diazotrophicus*, a nitrogen-fixing bacterium, has been isolated from coffee roots and coffee berries. Other members of the Rubiaceae, to which coffee belongs, have been shown to have bacterial endophytes. As the testing and isolation of these bacteria becomes more common, a specific endophyte might be advertised on packages to promote a taste it imparts to the bean.

corn (*Zea mays*). A study in Africa, where corn can be overwhelmed and outcompeted by the weed *Striga hermonthica*, showed growth improved when plants were inoculated with three different endophytes from the genera *Enterobacter*, *Klebsiella*, and *Pseudomonas*. This has great humanitarian implications and is deserving of further study.

lettuce (*Lactuca sativa*). The nutritional value of a bowl of lettuce is not what it was before World War II. Scientists are working to change that by using PPFMS, which are endophytic and help increase levels of vitamin B12 in lettuce, enough to meet the recommended daily allowance for it. Vitamin B12 is one of the substances needed by plants and animals but which most cannot produce; methylobacteria to the rescue.

potato (*Solanum tuberosum*). Potato seedlings show more root hairs when infected with endophytic bacteria, which results in better plants and potatoes. Inoculation of potato plantlets makes it possible to eliminate hardening-off (acclimation to UV radiation and wind after being started indoors); this is an expensive operation for potato growers who start under glass or indoors under lights, as with micropropagated plantlets. More root hairs also means more rhizophagy cycling. Finally, potato plants grown in

culture with pseudomonad bacteria had larger root systems and, most important of all, better yield. Endophytes are being further explored to increase the production of siderophores and antibiotics and reduce the incidence of *Streptomyces scabies* and *Xanthomonas campestris*, pathogens that cause scab and potato black rot, respectively, and lots of other diseases.

root vegetables. A study of potato, beet, rutabaga, Jerusalem artichoke, and carrot, which are often eaten raw (smoothies and macrobiotic diets, anyone?), show big Alphaproteobacteria populations in peel and pulp samples. Only potatoes had Saccharibacteria. Beets had more representation from the phylum Firmicutes.

soybean (*Glycine max*). Soybean already partners with nitrogen-fixing endophytic bacteria. Methylobacteria improve the amino acid profile of soybean seed, making a better soybean. This is a key cash crop, and lots of endophytic research centers around it.

squash (Cucurbitaceae). *Bacillus* is the number one genus of endophytes found in squash. As is seen in so many other plants, wild taxa have numbers and kinds of endophytes that differ from those found in cultivated taxa, which have been lost due to commercialization. Bacterial isolates show antipathogenic abilities when applied to plants. The identified bacteria may be useful for protecting plants, not only in the field but also postharvest.

strawberry (*Fragaria*). The presence of endophytes was confirmed after measuring endospheric production of auxin (IAA) and phosphate solubilization capability. Test plants had 20 *Bacillus* and *Sphingopyxis* strains. And when strawberry plantlets were inoculated with each isolate, seven of them were shown to increase plantlet root growth, length, and weight. Strawberry seed endophytes have also been manipulated to improve the yield and antioxidant content of strawberries. This sounds like something both commercial strawberry growers and home gardeners will be hoping works in the field.

TURF AND OTHER GRASSES

Endophytes do well in grasses, and grasses hold a special place in the hearts of endophytic researchers: it was the alkaloids produced by fungal

endophytes, which prevented grazing, that first drew attention to the presence of endophytes and their potential.

The world's reliance on grains and grasses for human and cattle consumption is so great, any endophytic bacterial impact on them is of keen interest. As a result, there are more products available for use with these plants than any others, and some of the discoveries for agricultural uses have been incorporated into seeds available for lawns, golf courses, sports fields, and other grassy venues. Look for labels that announce the seed is coated with endophytes.

Endophytes enhance growth by solubilizing phosphorus, and seeds coated with these bacteria are now available. This is good and timely news: phosphorus is now such a limited resource, it is no longer allowed, by law, in U.S. lawn fertilizers.

Seeding with grasses that contain endophytes (perennial ryegrass, tall fescue, hard fescue, Chewings red fescue, creeping red fescue) has been proven to reduce the need for pesticides to control aboveground chewing insects. Already, consumers in parts of the world are able to buy endophytically enhanced lawn seed, bred to ensure endophytic activity; this is the new way to control webworm (*Hyphantria cunea*). Turf grasses infected with the fungal endophyte *Epichloë festucae* did better against leaf spot pathogens *Sclerotinia homoeocarpa* and *Laetisaria fuciformis*. Now scientists need to find out why.

But perhaps the most exciting prospect (and it is hard to pick just one) for the use of endophytic bacteria is weed control—an area of study near and dear to me and my dandelion-filled lawn. Use of endophytes could change the herbicide game. A bioherbicide that is organic and safe and works from the inside would be very welcome—and it could pass its weed-fighting traits on via seeds. Although I have made my peace with dandelions (they won!), count me in with this development.

Among endophytes that simultaneously promote the growth of grass but kill off neighboring weeds, *Pseudomonas* and *Xanthomonas* are the most studied genera. *Xanthomonas campestris* pv. *poae* and *X. campestris* controlled annual bluegrass (*Poa annua*) and Canadian horseweed (*Conyza canadensis*), both of which can invade lawns, by producing allopathic metabolites that prevent germination (and sometimes growth). Some strains may not kill but at least suppress the growth of cheat grass (*Bromus tectorum*), green foxtail (*Setaria viridis*), and other weeds.

barley (*Hordeum vulgare*). One grass that is full of endophytes is barley. Scientists have attributed an increase in vitamin content, nutritional quality, and enzyme activity to the action of seed microbes in the grain, but which specific bacterial endophytes are involved is yet to be fully determined. The prime suspects? Actinobacteria, Bacteroidetes, and Proteobacteria are the main endophytic bacteria phyla in barley's rhizosphere and roots.

We know endophytic bacteria involved in rhizophagy increase a plant's resistance to cold. A great study project for grad students and scientific farmers would be to work with this grain crop, which is still experimental in my Alaska, and develop endophytes that improve barley's tolerance to cold.

sugarcane (*Saccharum officinarum*). This is not a plant many gardeners grow, but it is a grass of great commercial importance. One of the many studies involving endophytes and sugarcane identified 29 strains in its apoplastic sap alone. In another, young sugarcanes were cultured with a nitrogen-fixing strain of *Klebsiella variicola*. After they were hardened off, they developed better roots, shoots, and plants as measured by dry weight. My ears perked up at the increases in NPK, compared to controls. In another study on sugarcane the same endophytic bacterium was shown to improve nitrogen fixation.

TREES

It is hard to imagine a bacterial endophyte way up at the top of a sequoia. I haven't looked, but I am betting there are several. And who knew the health of our apple trees and spruces were dependent on endophytic bacteria, engaging in rhizophagy down in the roots and then making their way into the upper parts of these trees? Yet our friends in the phyla Actinobacteria, Firmicutes, and Proteobacteria (especially its genera *Acinetobacter* and *Sphingomonas*) all have reps that thrive in trees, and Actinobacteria are in fact found more frequently in forest trees than in agricultural crops. This has me imagining an endophytic bacterium that could help a tree ward off bark beetles. Some are no doubt in place, but climate change may be impacting them. We already have a few endophytic bacteria that can take care of caterpillars. What insect or pathogen is next?

olive (*Olea europaea*). The Big Three—Actinobacteria, Firmicutes, and Proteobacteria—are, no surprise, the dominant endophytic phyla in olives.

Olive genotypes are apparently quite selective about (not to say controlling of) their mix of endophytes, a recent study showed. Perhaps the next study, comparing Roman olive seeds to modern ones, will tell us how far back some of these same bacteria have been olive-endophytic.

poplar (*Populus*). Poplars are key trees for producing biomass around the world, and endophytes are being studied to find ways to increase the speed and amount of that production. Of the 75 or so bacterial endophytes collected from poplar and willow (*Salix*), Gammaproteobacteria dominated. *Enterobacter* sp. strain 638 consistently increases biomass.

Scots pine (*Pinus sylvestris*). *Methylobacterium extorquens* bestows positive beneficial effects, comparable with those seen by using ectomycorrhizal fungi *Suillus variegatus* and *Pisolithus arhizus*. It and they work together as well.

SPECIAL ON TOMATOES

Tomatoes are the number one plant grown by home gardeners. Both they and cannabis are also big commercial crops. Happily, these wonderful plants benefit from our new knowledge of endophytic bacteria and the rhizophagy cycle.

Let's start with tomatoes. Who doesn't love a great tomato? Nobody, apparently. Many of us start them every spring from seed. The rest buy a plant (or six) and proudly grow them through fruiting. Heirloom tomatoes, a strain of plant adapted to its locale, do particularly well because there is a location-specific set of endophytes among the mix in their (unsterilized!) seeds. Once the seed germinates, these bacteria are free to populate the soil, enter roots, and cohabitate with their host.

One research project isolated 120 different endophytic bacteria from tomatoes. *Bacillus* strains dominated. When tested on agar, antifungal and antibacterial properties were noted. When tested in plants, they promoted growth as well as produced antimicrobial compounds. *Bacillus amyloliquefaciens*, which we have seen before, was one of the more beneficial strains; it also happens to be commercially available.

Another research project isolated 49 endophytic bacterial strains in tomato roots alone, chiefly from the genera *Bacillus*, *Pseudomonas*, and *Rhizobium*. Most of the plant growth came from *Pseudomonas*; the 18

nitrogen-fixing *Bacillus* and *Rhizobium* strains, on the other hand, were better at fighting pathogens and defending the tomato. How do these endophytic bacteria get into their tomato plants? We know it is via roots (with seeds and soil as vectors), the considerable endophytic life in the xylem and leaves notwithstanding.

The endophytic bacterium *Pseudomonas fluorescens* deposits the antibiotic DAPG (2,4-diacetylphloroglucinol) in and around the roots of tomato. And *Micrococcus luteus* was found to promote tomato growth. After these spherical bacteria go intracellular, tomato roots show increased root hair length—rhizophagy, anyone? Endophytic bacteria isolated from tomatoes also helped wheat and other plants grow. And finally, endophytic bacteria are found in tomato trichomes, and recent work shows some engaging like rhizophagy cyclers. Do these bacteria cause trichome formation? There is much work to do but so many potential rewards, from which both farmers and home gardeners stand to benefit.

CANNABIS

Cannabis is a big industry, legal and not, worldwide. Plants were first grown for their fiber and seed; this non-psychoactive hemp, as it is called, has since been joined by varieties that contain psychoactive cannabinoids. Increasing use of cannabis fiber, seed, leaves, and flowers makes cannabis an ideal plant to consider growing with the assistance of endophytes. Why? Because, lo and behold, cannabis is a plant that hosts many bacterial endophytes—and endophytic bacteria themselves produce a lot of the chemicals found in cannabis.

The major phytocannabinoids in *Cannabis* spp. are delta-9-tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD), cannabidiol-carboxylic acid, cannabigerol (CBG), and cannabichromene (CBC); as well, terpenes and flavonoids are produced in special glandular trichomes (from the Greek, “growth,” “hair”). Bacteria can produce the same metabolites. Could endophytic bacteria be helping cannabis plants produce their phytocannabinoids (in addition to engaging in the rhizophagy cycle)?

Just as they do tomatoes, trichomes cover cannabis plants to protect against predators and harsh conditions, such as too much light. Are there bacteria in trichomes? Are these hairs formed similarly to the way root hairs are formed? Research has been limited because the plants have been

categorized as illegal. Access and study have been restricted, but as laws fall away, the pace of research is increasing—and if bacteria are involved with these trichomes, it will be explored in detail. (I am guessing they are.)

Regardless of how trichomes form, production of THC is influenced by soil microbiota. More research is needed, but it appears the concentration of cannabinoids is also related to the endosphere community. Time will tell if bacteria have even more to do with production of cannabinoids and flavonoids in cannabis, but reports of THC-producing bacteria are already coming in.

Cannabis endophytic bacteria play a crucial, positive role; they induce phytohormones, which must impact the plant's desired metabolites. Examples of endophytic bacteria making the same metabolites as their host plant abound. It makes sense when you consider bacteria and plants both need to protect themselves from some of the same enemies. In addition, endophytic bacteria really have an incentive to help their hosts. And even if an endophytic bacterium can't itself make a secondary metabolite that is similar to one produced by its host, it might trigger the plant to do so.

One study used several strains of cannabis plants to see if there were population differences between rhizosphere cannabis microbes and endospheric populations. Results suggest, again, that a core group of bacterial endophytes is used by cannabis: the genus *Pseudomonas*, the families Oxalobacteraceae and Xanthomonadaceae, and the orders Actinomycetales and Sphingobacteriales. This study showed the rhizosphere bacterial populations were the source of the endosphere's. Living soil and cannabis go hand in hand.

This same study showed the rhizosphere populations for all the different cannabis cultivars (selectively cultivated varieties) were pretty much the same, but the amount of endophytic bacteria in each plant was determined by the cultivar, and differed from cultivar to cultivar. This is a result of the evolution both host and invader must undergo to live together. Finally, the cannabis genotype appears to influence the endophytes in the plant. This has huge implications for growers.

Cannabis leaves host several endophytic *Pseudomonas* spp. and a host (pardon the pun) of *Bacillus*: *B. licheniformis*, *B. megaterium*, *B. pumilus*, and
B. subtilis. *Pantoea* and *Staphylococcus* strains were found only in petioles, and seeds carried *Bacillus, Enterobacter*, *Pantoea*, and *Staphylococcus*.

Roots, on the other hand, host various members of *Acinetobacter*, *Chryseobacterium*, *Enterobacter*, *Microbacterium*, and *Pseudomonas*.

Endophytes are tolerated by hosts because they control plant pathogens, increase disease resistance, and even modulate the production of secondary metabolites. So it makes sense that these endophytic bacteria help cannabis plants produce more and better product. Sure enough, when various endophytes known for growth-enhancing properties—*Azospirillum brasilense*, *Gluconacetobacter diazotrophicus*, and *Herbaspirillum seropedicae*—were isolated from corn, sugarcane, Bermuda grass, and sorghum and tested as a mix in cannabis, flower yield and plant height increased. There were also more metabolites, THC being one.

Again, it doesn't seem the soil community decides who gets to become endophytic. Rather the cannabis cultivar does, and each chooses a different mix. Of course, just like an heirloom tomato, why wouldn't Durban Poison, a landrace strain from South Africa, have its own unique set of endophytes in its seeds, different from those in Mexico's Acapulco Gold? Amazing as it is, we have to keep remembering the plant is in control.

There has been considerable effort in the cannabis industry to develop terroir, a sense of taste from the growing location, as in wines. Do specific endophytes help cannabis plants develop distinct flavors? As soon as research restrictions are fully lifted, we'll have some answers—and some biological products to use. We need to know more, but a good, diverse soil bacterial population allows any plant to choose what it needs, and some of what it needs are endophytic bacteria.

Seeds are a big vector for endophytic bacteria, and cannabis seed is no exception. If a seed has been sterilized, it will not have microbes from the mother plant. It won't grow as it is supposed to. You can do as much genetic work as you want to increase levels of THC and other cannabinoids, but paying attention to the genetics of the microbes in the seed may be just as useful, if not more.

Some home growers have problems getting cannabis seeds to germinate and survive. Poor or no germination is a big complaint, especially from the beginning grower. This can often be traced to seeds being soaked in hydrogen peroxide. Lore has it that this improves germination. No, it kills microbes and makes germination more difficult. This is a practice that has to stop.

Many growers start seeds in damp paper towels. Once the seed germinates, the bacteria in it go into the towel. You should consider planting the paper towel in the soil along with the seed.

Another problem is seed that is no longer viable. Following the advice of other books, growers keep their seeds very dry. But microbes in seeds actually need moisture; otherwise, they will go dormant and eventually die. This is so even during seed storage. You really don't want your seeds to dry out, so desiccants do not make sense.

And consider how your seeds are harvested. You want them to be at peak endophyte, so to speak. Do you simply collect seeds as an afterthought of drying your flowers? This is not how flowers (and the seeds they contain) ripen in Nature. Mimicking where and how the seed ripens and the flower falls can help ensure your seeds have all the right endophytes. I am enough of a soil food web geek to point out that seeds pick up additional important microbes when they or the flower hits the ground. Time for growers to experiment.

One more point. If you grow a plant in soil that has already supported cannabis, the suite of available cannabis-loving bacteria from which it can choose endophytes should be larger than if you start in fresh soil or soil that supported a different kind of plant. The standard rule used to be don't reuse soil. Not so. Reuse of soil (absent root pests) for successive crops is a good practice. It even probably makes sense to grow each strain in its own reusable soil.

And then there is the rhizophagy cycle. We know it is operating in cannabis roots because it has been observed. Even if hadn't, cannabis plants produce extensive root hairs, and so we would know it has rhizophagy endophytes. This must explain why increasing the bacterial mix in the soil results in increased THC content. Again, more work needs to be done. Bacterial endophytes offer a real opportunity for growers to differentiate, invent, and move the cannabis industry forward.

BIOCONTROL

Preventing plant diseases is extremely costly. Pathogens develop resistance; it is an effort to stay one step ahead. Endophytic bacteria produce metabolites that are capable of working as herbicides and pesticides. Plants use these endophytes for survival. They can defend their hosts or outright attack competing plants nearby. Bioherbicide- and biopesticide-producing

endophytes obviously have a potential role in our efforts to deal with crop-reducing microbes, plants, and animals. They may even stay ahead of evolving pathogen resistance.

ENDOBIOME INTERFERENCE

Endophytic bacteria move into soil and can impact neighboring plants by using up key nutrients. Some produce siderophores that chelate metals—critical iron, for example. This is endobiome interference. It occurs when endophytes from one host are moved to a new type of plant or soil, where they disrupt the existing microbes or even competing plants (and sometimes both).

One promising experiment put microbes from different host plants—rosary pea (*Abrus precatorius*), snakecotton (*Froelichia gracilis*), tomato (*Solanum lycopersicum*), and annual bluegrass (*Poa annua*)—onto sterile seedlings of dandelion (*Taraxacum officinale*), curly dock (*Rumex crispus*), and clover (*Trifolium repens*). The bacteria used were *Curtobacterium*, *Micrococcus*, *Paenibacillus*, *Rhodococcus*, and *Sphingomonas* spp.

In their original host seedlings, none of the microbes negatively impacted growth or were pathogenic. After three weeks in the new seedlings, *Micrococcus luteus* and *Sphingomonas* bacteria slowed growth in their hosts. The reason has not yet been deciphered, but it is surmised the plants were overwhelmed by strange bacteria they could not control and to which they had not developed a tolerance.

Meanwhile *Curtobacterium* spp. turned out to promote growth in all three test species. Root mass was increased and mortality decreased. This is the opposite of endobiome interference. It doesn't really need a name; it's just another endophytic partner helping out its host plant in the way endophytes do.

MANAGEMENT PRACTICES

Knowing how the soil food web operates has already changed how gardeners grow plants and farmers cultivate crops. Rototilling and deep soil disturbance is no longer recommended. We use appropriate mulches, including composts. "Organic only" is the rule, of course. What other things should we be doing (or not doing) now that we have expanded the role of bacteria in the soil food web's functions?

It is clear that endophytic bacteria play a huge role in keeping plants healthy and helping them grow. Obviously, then, it is incumbent on growers to make sure growing practices don't harm them or interfere in their operation. There are even practices that will promote them.

Rototilling, use of artificial chemicals, and many other management practices are harmful to endophytic bacteria. We need to continue to avoid such practices. We also need to grow plants in ways that don't harm the endophytes we and they need.

Start with living soil: soil is where plants grow microbes. This is the pool for the population of endophytic bacteria that move into the plant. You have learned all the things they can do for your plants, so you probably intuitively know that you need to treat your soil well if you want to treat your endophytic bacteria well. You want to maintain the finest condominium for microbes that is possible.

ROTOTILLING

Gardeners don't like to hear that rototilling is no longer a recommended practice. Still there are lots of reasons. It is bad for soil structure. When you cut a worm in half, you don't get two worms. And when you till soil, you move what would become endophytic bacteria to new locations that are not necessarily where they should be. (Don't forget what happens to fungi and the other members of the soil food web when you disturb soil.) Displacing bacteria is not a good result, so it is not a good practice. The bacteria you displace might have been the ones that engage in rhizophagy with your crops.

CHEMICALS

Chemicals can obviously have negative impacts on endophytes. Organic is the only way to go if you want to protect them. The rule is to test inputs to make sure your addition is not harming microbes. (A before and after soil microbiome test will help.)

Antibiotics in soil and compost can be a problem for our endophytes which are, after all bacteria. Antibiotics kill specific bacteria. You can speculate what an application of antibiotics might mean for endophytic bacteria—the antibiotic might not impact the endophyte you wish to utilize—but testing is the only way to confirm if a particular agent will harm your

endophytes. Again, information is power. Growing controls with untreated soil is another way you can really know if inputs compete with endophytic bacteria.

Be careful using composts made with horse, cow, or chicken manures that contain antibiotics. Antibiotics should not be heavily used in animals, in any case. There is too much of an opportunity for resistant strains to develop.

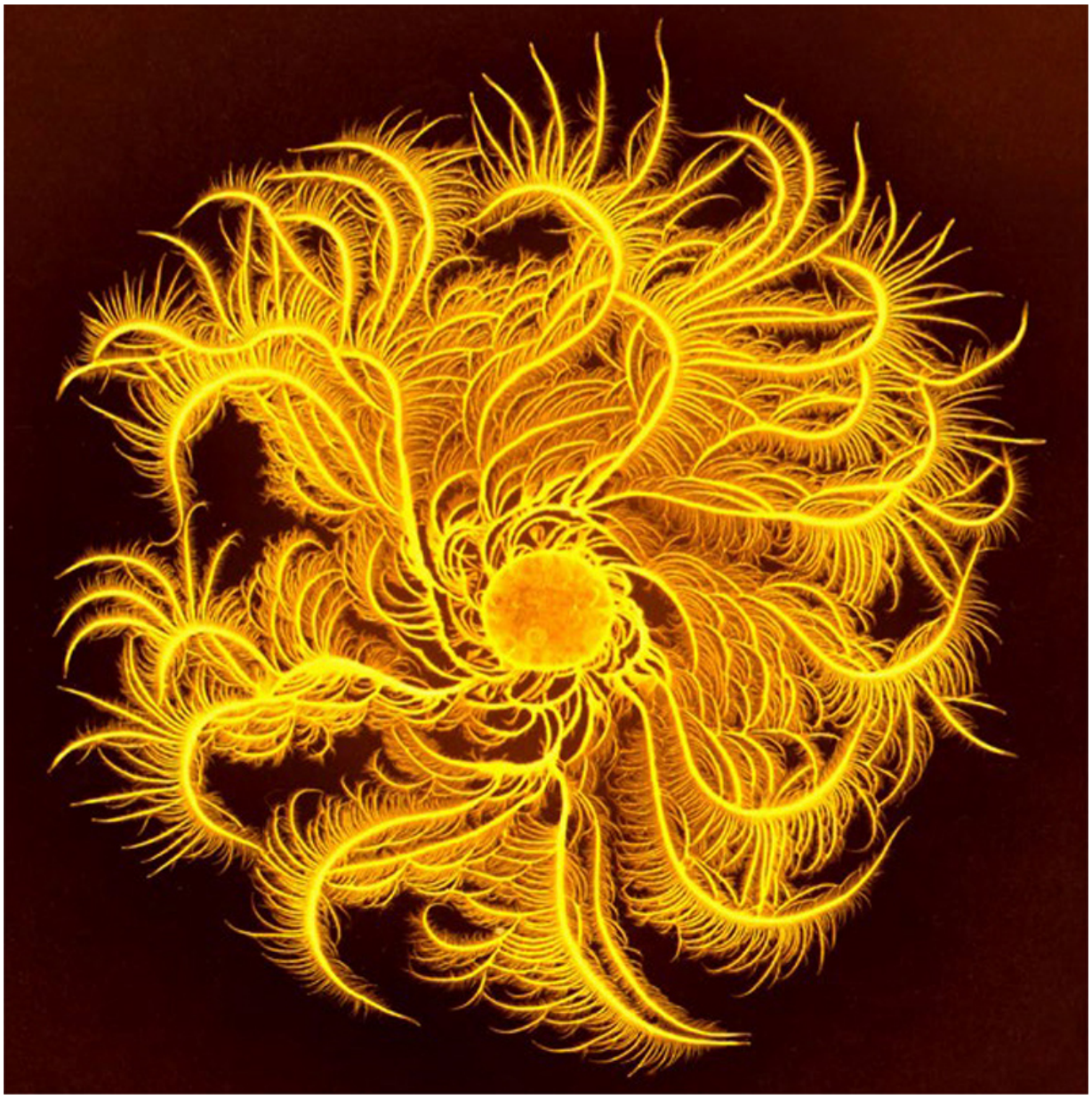
Pesticides alter endophyte activity too. So do herbicides. Endophytic bacteria sound like a better thing to use to control pathogens and other pests.

STERILIZING SOIL

I remember my father's soil sterilizer, a big box that superheated soil with steam. I inherited the beast but didn't bring it to Alaska because it was too big. My wife regretted that decision, as I tried to sterilize soils in the microwave instead. Even if sterilized soil made sense—which, for endophytic reasons and more, it doesn't—I will never do that again. What a stink.

In lieu of sterile soil, gardeners often use peat, which may or may not be sterile, as it turns out, depending on its prepackaging treatment. In any case, it would stand to reason that not only should you avoid sterile peat (if not peat altogether, for environmental reasons), but if you must use peat, mix it with living soil or compost or compost tea to ensure a good microbial talent pool, as I like to say, full of bacteria the plant can choose to use.

The same goes for coco coir, perlite, and vermiculite. They may not be sterile, but just in case, mix them with living soil or compost to ensure you have at least some rhizospheric (and thus endophytic) bacteria.



Paenibacillus sp.

Soil-less mixes are often sterilized during manufacture. Even if they have not, they surely don't have bacterial populations like those in real soil. What else need be said? With living soil there is a base population of bacteria from which the plant can choose. Not so with dead, soil-less mixes.

REUSING SOIL

Commercial growers of potted plants from poinsettia to cannabis fear losing their crops to pathogens and so usually don't reuse soil. The idea is that

over time, soil picks up harmful organisms, develops salts, and loses nutrients. Replacing soil is an expensive proposition. It surely doesn't fit with the level of regenerative agriculture we should be aiming for, but it is thought to lower the risk of a diseased crop.

Yet we know a living soil has great biodiversity. Do not toss your soil after growing a crop (unless it has root pests). Think about a cannabis or tomato plant spending its life in a 5-gallon container. It puts out exudates from the seed stage to post-flowering in order to maintain a proper herd of microbes. These don't disappear after the tomatoes are harvested and winter comes. They form endospores and otherwise survive the winter in the soil (and, yes, seeds).

Sure, you may need to test for and add some nutrients (and sometimes a root pest cannot be eradicated), but the presence of specific exudate-grown microbes at the time of starting the next crop has to be beneficial to it in so many ways. The pool of potential endophytes is deeper, the siderophore activity is greater, and the rhizophagy cycle will be that much easier to kickstart. It just doesn't make sense to blindly toss soil just because it has been used for a season. It did when this soil was full of chemicals and lacked microbes. Now, we grow organically.

It is not just the microbes and exudates located in used soil. It is the organic matter already breaking down and the endophytic bacteria that can get an immediate start neutralizing fungi and feeding the plant via the rhizophagy cycle. And if you leave old roots in the soil, the new plant roots will grow in the former's tracks, which requires less energy.

STERILIZING SEEDS

All manner of authoritative sources advise sterilizing seeds before planting. Home gardeners are warned that unsterilized seeds are vectors for foodborne pathogens like *Escherichia coli*, *Listeria*, *Salmonella*, and *Staphylococcus aureus*. The University of California—Davis suggests "heating [seed] on a stove for 5 minutes in a solution of 3% hydrogen peroxide (available in grocery stores) at 140°F" and then rinsing them in running tap water for a minute.

Sterilizing agents for seeds include hydrogen peroxide, which (even diluted) is going to be stronger than the ROS used to strip walls off rhizophagy bacteria—so how do you think your seeds' bacteria fare? Vinegar and diluted bleach solutions, even grapefruit seed extract—all are

recommended to remove pathogens. In all cases, the good bacteria go with the bad.

And yet we know endophytic bacteria engaging in the rhizophagy cycle cause root hairs to form. And not only that, but they help roots grow downward. Sterilize seeds and you risk a plant not even germinating, as the endophytic and epiphytic bacteria are often needed to degrade the seed case. Sterilizing seeds is a practice that should stop immediately.

COATED SEEDS

Seeds are often coated with fertilizers, clay, pesticides, and even rhizobia. Some of these coatings are designed to get a germinated seedling off to a great start nutritionally. Others increase the surface that comes into contact with soil. These seed coatings work or they would not remain on the market. However, just because they work doesn't mean they don't impact endophytes. Unless you or someone else does research and testing, it is impossible to tell if the plant would have been better off had the seed been uncoated. The bottom line? Test yourself or work with someone who has done testing.

STORING SEEDS

How you store seeds is very important. Many growers store them in an airtight container with a packet of desiccant. This is not what someone who understands endophytic bacteria would ever do. The endophytic microbes living on and in each seed coat need water to live. Suck it out of the microbe's environment and you kill it or perhaps turn it into an endospore, which will need time to reactivate. Time is money in a grower's world.

Much research is needed to see what happens to seed endophytes in storage, in order to make sure we are not limiting their effectiveness.

COMPOST VS VERMICOMPOST

The dominant bacterial phyla in compost are Actinobacteria and Firmicutes, and *Acetobacter* and *Lactobacillus* are the two biggest genera. The high temperatures reached in making compost encourage lots of Gram-negative bacteria like these, and remember: when it gets too hot, only Gram-negative bacteria form endospores and survive to live in the final product. The dominant bacterial phyla in vermicompost are Acidobacteria, Bacteroidetes,

and Gemmatimonadetes, and the same two genera are prominent. Vermicompost is the result of a biological process, not a thermal one. Earthworms ingest organic matter in their quest to devour a few protozoa (and maybe a few bacteria as appetizers).

Worried about killing bacteria during the thermal composting process? Tobacco plant rhizosphere grown in autoclaved soil has the same colonization by fluorescent pseudomonads, actinomycetes, and other bacteria as soil that was not heated. It is the fungi that take a while to come back, actually.

The choice of whether to use thermal compost or vermicompost might be dependent on what kind of endophytic bacteria helps the plants destined to be grown in it. Is it possible to match up one or the other type of compost with a particular crop? Clearly, this is another subject to study.

Since amateur gardeners are not yet able to readily identify species of bacteria or fungi, a good practice is to grow in living soils so the plant can choose what it needs. This is sort of how evolution operates. It usually makes sense to just let plants work wonders in selecting what is needed. The rhizophagy cycle doesn't change that aspect of using the soil food web to garden.

COMPOST TEAS AND MULCHES

Studies have confirmed that there are three zones in the soil around cultivated plants. The outer zone is the wild, undisturbed soil with generalized microbial populations. Next is the area influenced by exudates; this has a more selective microbiome. The zone inside the plant, the endobiome, has even fewer types of bacteria, all drawn from the soil.

While it is extremely difficult for a gardener to isolate individual endophytes, it makes sense to consider what goes into compost, bokashi mixes, KNF formulations, and compost teas. If you are growing cannabis, why not make your compost with as much dead cannabis material as you can? Spraying your lawn with compost tea made from compost that was heavy on grasses taken from the lawn makes sense when you want to garden with endophytes. This is a sort of recycling: recycle bacteria by using products they are in to make new products.

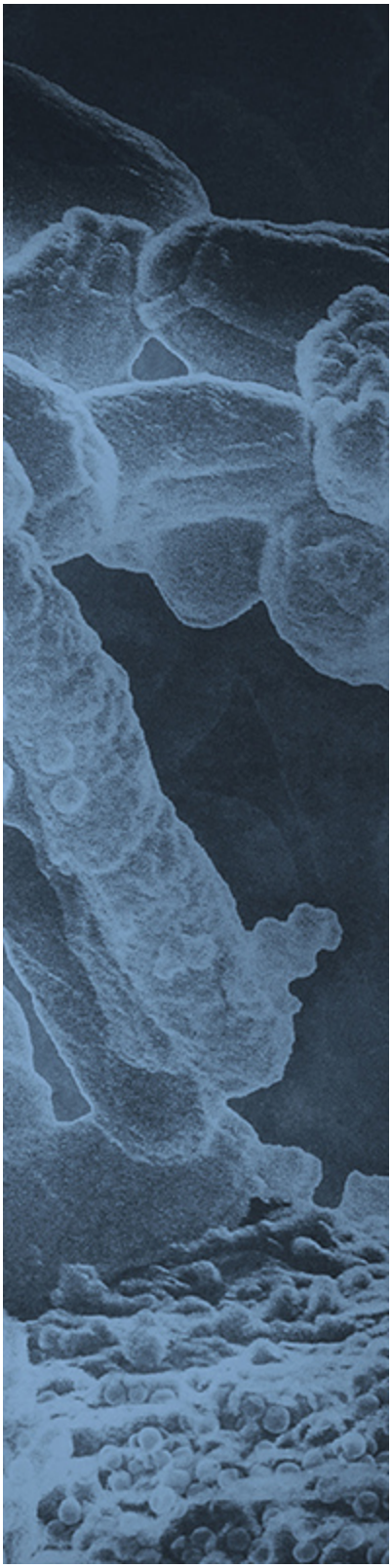
As more and more investigation results become available, we can learn which mulch material has an endobiome close to that of the plant being mulched. There will be charts developed to help. We won't have to isolate a

thing, literally just read which phyla (and even species) a particular plant associates with. As a hypothetical, barley straw mulch might really boost, say, strawberries because of an overlap of endophytic bacteria phyla in both. (Good thing I listed some of these associations so you can start to look them up for yourself.)

Use of compost teas should now be put in context with the rhizophagy cycle, which requires a healthy supply of bacteria to function efficiently. Those who find teas do not show any difference in growth or plant health may already have soils with enough rhizophagy bacteria. When the differences between control plants and those grown with compost teas *are* quite visible, is this because your soil was deficient in rhizophagy bacteria, now provided by the teas? There's only one way to find out: try a compost tea yourself and see.

STAY THE COURSE

The rhizophagy cycle is not a new system, only one that has been recently discovered. It and endophytic bacteria have always been part of the soil food web. They do not require a whole new set of gardening rules but rather that the grower continue to think in terms of protecting soil life, increasing microbial diversity, and allowing the plant to be in control. The basic soil food web rules still apply, in other words. Meanwhile, this is one of those times when our developing knowledge has left us with the cart before the horse. The new home testing methods that are coming (soon!) will allow us to wield our new knowledge precisely, so we can use the right endophytic bacteria on the right plant.



The Endophytic Future

WE HAVE KNOWN about endophytic bacteria for a while now, and some farmers and gardeners are already using available endophytic products on their crops. But many more folks have yet to be introduced to endophytes, and practically everyone needs to learn about the rhizophagy cycle. I see a great future for both.

Lest we forget, humans are mostly made up of bacteria, endo ones at that. It is estimated some 35 trillion bacterial cells are thriving among our mere 30 trillion human cells. We know bacteria have a profound impact on us. Why wouldn't they have as great an impact on plants? Bacteria rule.

RECOGNITION

It is time the world takes note of the rhizophagy cycle (and it will). The soil food web, considered a crazy idea in 2006, has become a universally accepted concept. (Thank you, Elaine Ingham.) If we could have seen these bacteria back then, they would have been part of the explanation of how plants obtain necessary nutrients via microbes. You have just been presented with an additional piece, the rhizophagy cycle. (Thank you, Chanyarat Paungfoo-Lonhienne and James Francis White.) The nitrogen cycle and the carbon cycle are now standard educational fare, and the rhizophagy cycle will definitely join them on that roster. It's only a matter of time before there is a general understanding of this newly visible, probably ancient, natural cycle.

How amazing are the dyes and stains that allow scientists to visually capture and distinguish the presence of hydrogen peroxide and ethylene? Or that nitric acid is being produced intracellularly and is converted to plant-usable nitrate? We can now see the waves of bacteria coming from the tips of the same plants we thought were sterile.

Experiments and studies thus far on the subject have unraveled many of the mechanisms in play. We can now say that bacteria—not just those living in the soil, but some residing inside the root—are feeding plants. It is not just scientists who need to catch up with this new branch of the soil food

web; so do thinking farmers, growers, and gardeners. We are all on the same journey, all with similar goals.

Plants herding bacteria is not a new idea; herding them intracellularly, however, is new. And while videos and confocal laser microscopy show it exists, I am the first to admit that just as with the soil food web in 2006, it is an invisible world, and we laypeople have to rely on the scientific community. Some of what we think we already understand will turn out to be wrong or will be supplemented by new discoveries. No matter. The rhizophagy cycle is a fact of life. It is time to move forward and figure out how to ensure it works optimally. This will happen.

EDUCATION

While endophytic bacteria are receiving recognition among some scientists, it is basically only soil scientists around the world who have been exposed to the idea of the rhizophagy cycle. My guess is you now know more about rhizophagy than most scientists outside of the field. This points to the very first step that needs to be taken: a massive educational effort.

Endophytic bacterial products are coming. Some are already here for those in the know. As for the rhizophagy cycle, it may not be fully understood, but there is enough of a framework to use it to help us grow plants more efficiently and sustainably.

Given the pace of research, I promise you will be hearing more and more about endophytes and rhizophagy. Using gibberellic acid was novel when I was a kid. No one knew about its powers. Today, growers all around the world are using it to enhance harvests. It is time for the same thing to happen to endophytic bacteria and the rhizophagy cycle.

BIOBASED SYSTEMS

We can no longer afford practices that, while allowing us to feed the world, destroy it. We are losing soil rapidly and applying way too many pesticides and herbicides to be sustainable. These are damaging enough in their own right, causing a devastating loss of insect diversity, including pollinators, not to mention the added dangers to humans associated with application methods. Large- and small-scale growers are looking for biological solutions. The time will come when soil loss and climate concerns will make these mandatory. No matter what we call it—regenerative agriculture,

biodynamics, soil food webbing, or something else denoting a sustainable, organic system—we know we need to treat our planet better by growing smarter and with a much-reduced use of chemicals. This is where endophytes come in. They can take up the jobs harmful chemicals now do. They will be a huge part of the solution.

Even without the nutrient benefits plants derive from the rhizophagy cycle, we know bacterial endophytes can help plants gain nutrients in other ways (siderophores, reduced competition from fungi, etc.). Already farmers use some commercially available endophytic formulations and not just nitrogen-fixing products and Bt. Many of these products will have the same impact in home gardens, whether that be increasing antioxidants in strawberries, vitamin B12 in lettuces, or just growing more peas and beans.

Gardeners must actively seek out the new endophytic bacterial products farmers are using. Step one is to set a few alerts with your internet search engine. Enter “endophytes” and “rhizophagy,” and you will be alerted whenever a new discovery is made or a product employing endophytes becomes available. Step two is to speak with your local nursery to make sure they are staying up to speed.

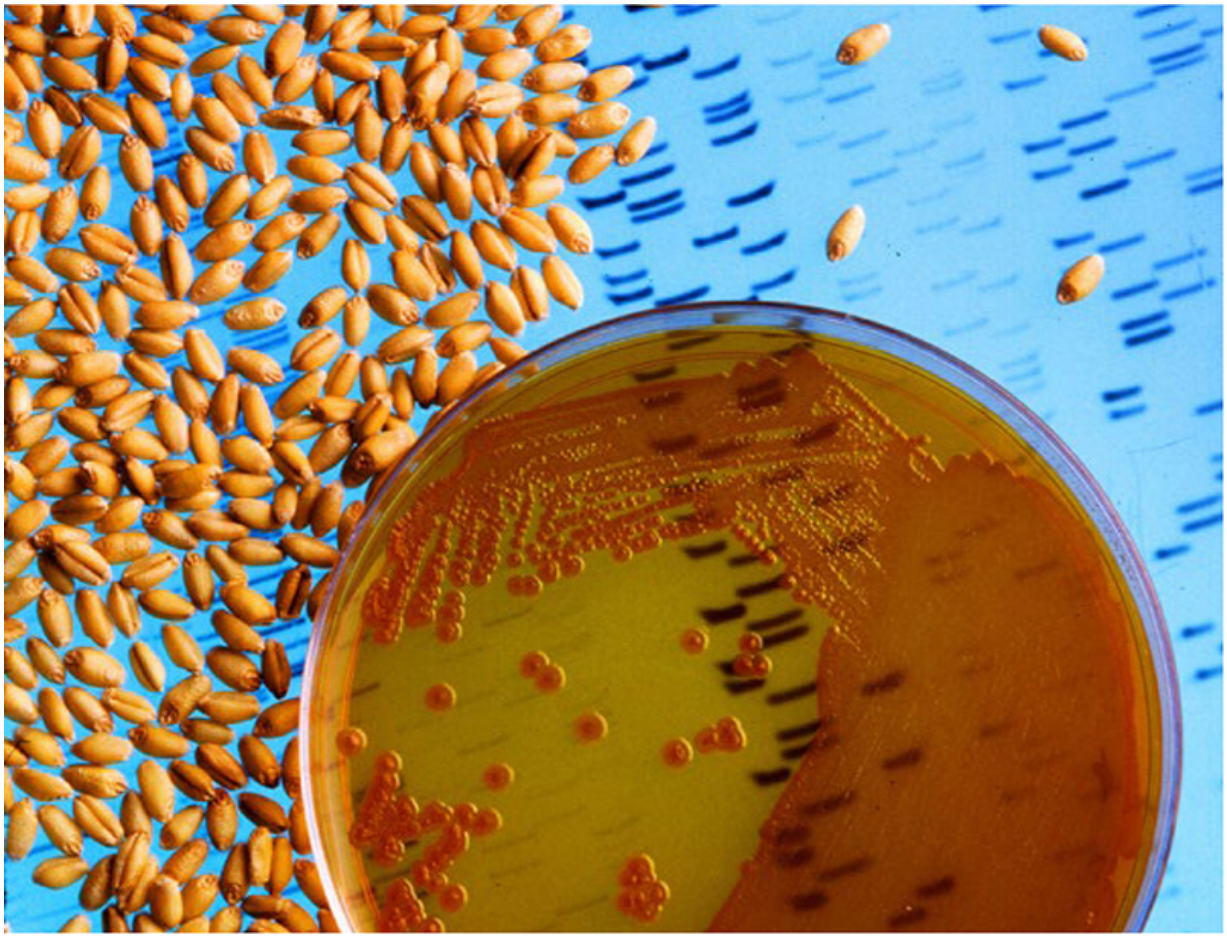
NEW DEVELOPMENTS

New endophytic bacterial products will hit the market, packaged for both large- and small-scale growers. Many of these products will be plant- and pathogen-specific: that is the nature of endophytic bacteria which, you now understand, are very particular about choosing where and with whom they live. Still, with eyes on the bottom line, efforts will be directed toward making them applicable to as broad a plant spectrum as possible.

Some of these products will be put on seeds, others mixed in liquids for spraying or soaking the soil or adding to irrigation systems. They will be developed to fit in with existing delivery technology, but new application methods will inevitably arise. These are live bacteria, capable of multiplying at fast speeds and working from within the plant. Having read this book, you should be able to see it all coming. Now that you’re almost finished, look closer and you will see it is already happening.

Be ready for an endophyte that takes out dandelions or crabgrass—these markets are too big for commercial developers to pass up. How about preventing chickweed seedlings from competing with your vegetables? Or

finding endophytic bacteria that make cover crops even more effective? (Perhaps you will never need to fertilize again.)



Seeds will be coated and used as vectors for endophytic bacterial products.

And just think what is happening in the field of microscopy. Who could have imagined actually being able to see DNA strands? There are so many different new ways to look at something and build a true picture of what is going on. I am not sure if we will see home versions, but some of these better microscopes will be common in high schools and universities.

You can expect all this to tie in with your cell phone. What gardener could have imagined the macro lens cameras some cell phones already contain? We will see microscopy advances applied to cell phone cameras or perhaps attachment lenses that will help us test for, see, or otherwise ID bacterial endophytes. And, of course, beyond cell phones, there are digital cameras and DSLR cameras. All information obtained can be linked into those huge international databases for bacterial identification.

A CHANGING WORLD

The world population is predicted to top eight billion in 2025. All these people need food, and it is going to take a better system to ensure demand is met as our population grows even larger. Biofertilizers are clearly going to be part of that system, and endophytic bacteria are high up on that list. Once inoculated, plants and their endophytic bacteria will produce much of their own nitrogen and other essential nutrients, and they'll do it in a more efficient way.

We are running out of phosphorus. We faced a similar situation in 1898, only then we were running low on nitrogen; that challenge resulted in the development of the Haber-Bosch process, whereby nitrogen is artificially fixed. Some endophytic bacteria are phosphate-solubilizers as well as nitrogen-fixers, potash-mobilizers, and siderophore zinc-boron-sulfur-solubilizers. Bring them on (actually, in).

The market is demanding organic food for health reasons as well as for environmental ones. Endophytes fit this need too. They provide fertility, promote plant health, and foster agricultural sustainability naturally. They replace expensive and energy-intensive processes.

Climate change is already changing growing zones globally. Lucky for us, endophytes that allow us to grow crops in unprecedentedly warmer growing zones have already been isolated. And the prospects for use of endophytes in commercial agriculture are extremely promising. Small research projects are morphing into companies set up to commercialize endophytic discoveries, and huge companies are developing and selling inoculants (not that you have to be huge to be good). These include Novozymes (Denmark), BASF (Germany), MBFi (South Africa), and DuPont, Horticultural Alliance, and Soil Technologies Corporation (U.S.).

It isn't just chemical fertilizers that are harmful to the environment, ruining our soils and polluting our waters. Our chemical attempts to control invasive weeds don't belong in the environment either. Using endobiome interference, endophytes can repress weed growth; the subject plant may even grow better while endophytes are on the job, giving any competitor species some real competition. This is a beauty of endophytic bacteria. You can use more than one kind, and they don't seem to interfere with each other.

LEGISLATION

Of course, until the world is fully educated as to their safety and value, there may be problems using biofertilizers, biopesticides, and bioherbicides. Regulators worried about concentrations of harmful microbes already mistake some endophytes for pathogens. (Just ask anyone in the cannabis industry.) This cannot be allowed to happen. Not all bacteria are bad bacteria. To the contrary, right?

In July 2017, the U.S. Department of Energy funded the Center for Bioenergy Innovation at ORNL to accelerate biofuels and bioproducts research.

We have to keep an eye on legislation, to make sure nothing adverse is imposed on the industries developing endophytic microbial products. In the main, such an imposition comes from ignorance of the science of endophytic bacteria. If you don't understand what they are and how they work, then it is not possible to deal with endophytic bacteria in a sensible manner. But adverse legislation and regulation can also stem from the opposition—industries who see endophytes as competition. They have the money to lobby; the gardener does not. Gardeners have the numbers, however, and should not allow this or any form of greenwashing. Stay vigilant!

Speaking of following the money: it's important to ensure there are ample amounts of grant monies to fund the research that for-profit companies will not undertake. These studies will pay for themselves in savings as we shift to more sustainable and efficient agricultural practices. The more we know about endophytic bacteria, the better off our food supply, the cleaner our waters, and the healthier our planet.

MARKET SPECIALIZATION

To each his own, so to speak. The more we learn about the selectivity between plants and endophytic bacteria, the better we will be at developing specialization in the formulation, manufacture, and application of them. In addition to studying endophytes directly, some scientists are developing procedures to enable us to better isolate and test endophytes; with that accomplished, we can make better guesses at starting points and speed up determination of benefits.

Specific endophytic bacteria are now being used on rice and cereal crops all around the world. Bioproducts that pair up with vegetables and specific crops, including cannabis, are increasingly common. In fact, the bacterial inoculant segment of the agricultural market already leads the global market of biofertilizers with a 65.7% share in 2017 (the most recent year for which data is available). Sales of bacterial inoculants are expected to increase by approximately 10% annually over the next few years.

And beyond the part they play in growing our plants, endophytes continue to thrive in fruits and seeds, postharvest. It will be of immense value to have a few commercial endophytes riding along, preserving those cucumbers, squash, strawberries, and more as they travel from farm to tummy.

ENDOPHYTE-INFECTED SEED

Guaranteed, seed endophytes will be present in the next generation of seeds for sale, recognized as being indispensable for all things "seed," from collecting and handling to packaging and storing. Endophyte coatings will enhance growth and improve nutritional values. They will help more seeds germinate, and quicker—perhaps even under adverse conditions such as colder soils (for an earlier start). For the advanced rhizophagy endophyte gardener, there will even be classes on seed inoculation and techniques to

infect plants when flowering, to introduce endophytes into ovaries as they develop into seeds. Already there are studies of specific seed endophytes in sunflowers and other annual crops that gardeners grow, as well as a few on using bacterial endophytes for coffee trees—for those who want to drink the ultimate organic brew.

HOME DNA TESTING

Not too far in the future, home gardeners will be able to obtain affordable genetic testing of plants for endophytes. (The databases are already here.) We now have companies that read our own DNA, letting us know about potential illnesses; and some people, gardeners even, test their cat's and dog's DNA. You can, in fact, have a commercial lab do DNA testing on plants for you now, but it is an expensive proposition for a small hobby vegetable garden. This will change as demand develops, and this will happen as more gardeners, aware of the benefits of endophytic bacteria, want to know which plants have which endophytes.

NOT THE END

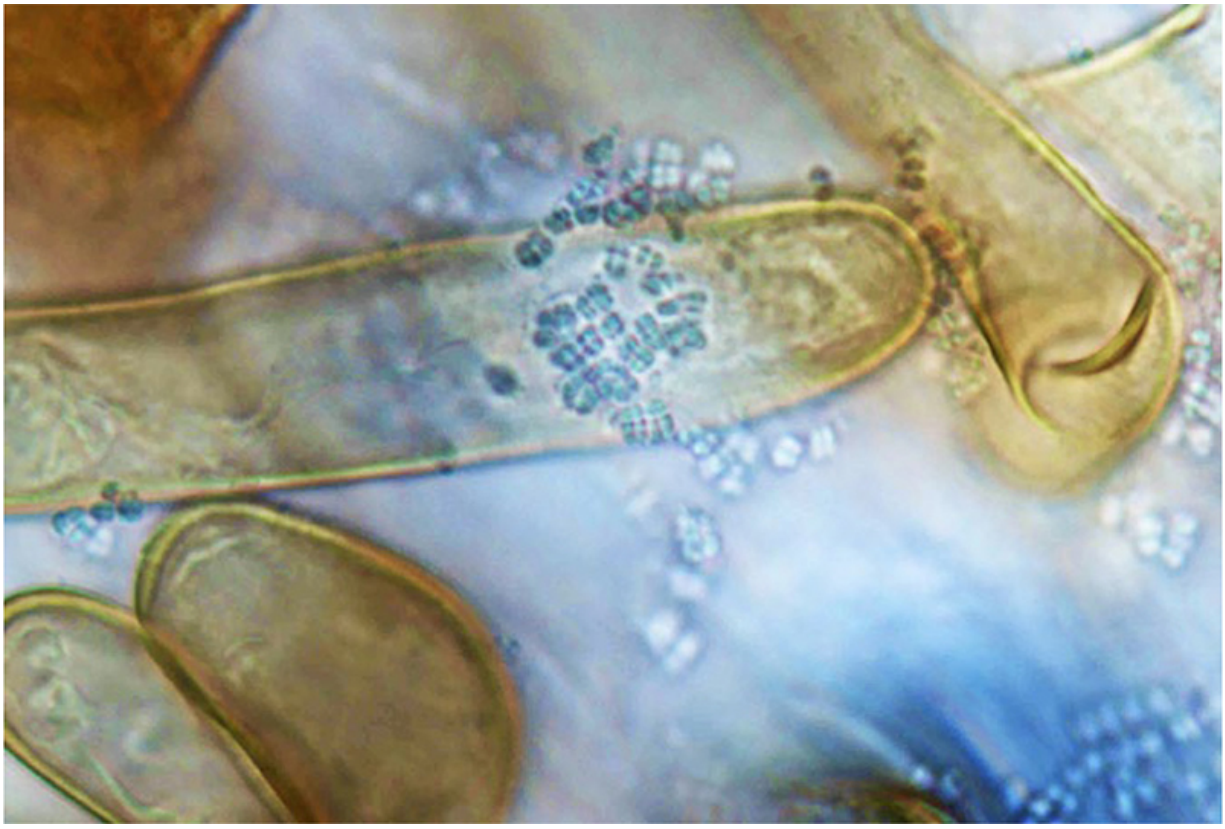
This book has taken you a little bit into the future. I hope it makes you a better grower of your plants right now and that you appreciate them a lot more. Your plants are in full control of their destinies, and they are not alone in this: they are joined by another master of the universe, bacteria. Plants and their endophytic bacterial cohorts know what to do and when to do it. We gardeners should get out of the way more.

As for the rhizophagy cycle, who would have thought it possible? You now know plants take up nutrients in inorganic form from the soil, yes, but also that they ingest organic (and living) bacteria as another way to obtain necessary nutrients. The soil food web may work just a bit differently than we thought back in 2006, but the plant is still very much in control.

I implore you to pause while gardening every now and then, lean on your winged weeder or push mower, and just contemplate the presence of endophytes in your plants. Think about all they do. Perhaps when you tend your carrots you will give a passing thought to those L-form rhizophagy bacteria, hurtling out of newly forming root hairs into your garden soils, rebuilding their walls, and then cycling though the carrot roots every two days until harvest. Who knew?

Finally, you have been exposed to loads of new things to think about (and some new words to look for the next time you scan a product label). Realize this is just the start of our understanding of endophytic bacteria and the rhizophagy cycle. I wonder what else we will learn about the workings of the soil food web as science moves forward.

Stay tuned.



Wonder of wonders, the soil food web—now including endophytic bacteria.

Resources

A Summary Chart

Some common endophytic bacterial genera and the plants they benefit as reported in literature (including Naveed et al. 2014a, 2014b; Rangjaroen et al. 2017).

Plant	**Endophytic bacterial genera**
banana	Azospirillum, Bacillus, Citrobacter, Enterobacter, Herbaspirillum, Klebsiella, Pseudomonas, Rhizobium, Serratia
cannabis	Acinetobacter, Azospirillum, Bacillus, Chryseobacterium, Enterobacter, Gluconacetobacter, Herbaspirillum, Microbacterium, Pantoea, Pseudomonas, Staphylococcus
carrot	Agrobacterium, Bacillus, Klebsiella, Pseudomonas, Rhizobium
coffee	Azospirillum, Gluconacetobacter
cotton	Azospirillum, Bacillus, Pseudomonas
cucumber	Agrobacterium, Bacillus, Chryseobacterium, Curtobacterium, Enterobacter, Micrococcus, Paenibacillus, Pseudomonas, Serratia
grape	Comamonas, Enterobacter, Klebsiella, Pantoea, Pseudomonas, Rhodococcus
maize	Achromobacter, Agrobacterium, Bacillus, Curtobacterium, Enterobacter, Herbaspirillum, Microbacterium, Micrococcus, Paenibacillus, Pseudomonas, Rhizobium, Serratia
pineapple	Azospirillum
potato	Acinetobacter, Agrobacterium, Bacillus, Chryseobacterium, Comamonas, Curtobacterium, Enterobacter, Klebsiella, Methylobacterium, Micrococcus, Paenibacillus, Pantoea, Pseudomonas, Serratia
rice	Agrobacterium, Azoarcus, Azorhizobium, Azospirillum, Bacillus, Bradyrhizobium, Enterobacter, Herbaspirillum, Klebsiella, Micrococcus, Pantoea, Pseudomonas, Rhizobium, Serratia
soybean	Agrobacterium, Bacillus, Bradyrhizobium, Enterobacter, Klebsiella, Pantoea, Pseudomonas
strawberry	Bacillus, Sphingopyxis

sugarcane	Acetobacter, Azospirillum, Bacillus, Gluconacetobacter, Herbaspirillum, Klebsiella, Pseudomonas
tomato	Escherichia, Pseudomonas
wheat	Azospirillum, Bacillus, Herbaspirillum, Klebsiella, Micrococcus, Pseudomonas

Endophytic Products

BASF / agriculture.basf.us
Bayer / cropscience.bayer.us
Bio-Care Technology / bio-caretechnology.com
Bioline / biolineagrosciences.es/#starskii
Bioworks / bioworksinc.com
Horticultural Alliance / horticulturalalliance.com
Mammoth / mammothmicrobes.com
Marrone Bio Innovations / marronebio.com/products/stargus
MBFi / mbfi.co.za
microBIOMETER / microbiometer.com
NewLeaf Symbiotics / newleafsym.com
Novosymes / novozymes.com
PGG Wrightson Seeds / pggwrightsonseeds.com/science-and-innovation/endophytes
Soil Technologies Corporation / soiltechcorp.com

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Index

Page numbers have been removed from the index for this electronic edition. To locate any of the following terms, please use the search feature of your e-book reader.

A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S | T | V | W | Y | Z

abiotic (non-biological) stresses
Abrus precatorius
abscisic acid
Acetobacter
Achromobacter
acidic hormones
Acidobacteria
Acidobacterium capsulatum
acids
Acinetobacter
Actinobacteria
Actinomycetales
actinomycetes
active transport
aeration
aerobic soil bacteria
agricultural biocontrol
Agrobacterium
alder
algae
alkaline
alkaloids
Alphaproteobacteria
Amaranthus

amino acids
ammonia
ammonium
anaerobic bacteria
angiosperms
annual bluegrass
antibiotics
antifungal compounds
antimicrobial compounds
antioxidants
apical meristem cells
Arabidopsis thaliana
Archaea (domain)
archaeans
arginine
asexual reproduction
Asian greens
aspirin
atoms
attachment pili
autoinducers
autotrophs
auxin
Azoarcus
Azorhizobium
Azospirillum
Azospirillum brasilense
Azospirillum formosense
Azotobacter
Azotobacter chroococcum

Bacillus
Bacillus amyloliquefaciens
Bacillus azotoformans
Bacillus coagulans
Bacillus firmus

Bacillus licheniformis
Bacillus megaterium
Bacillus pumilus
Bacillus subtilis
Bacillus thuringiensis (Bt)
subsp. *israelensis*
subsp. *kurstaki*
subsp. *kyushuensis*
Bacteria (domain)
bacteria
behavior of
capsule
cell walls
classification
combinations
cytoskeletons
decay
flagella
genera
measurement
movement
nomenclature
nutrient acquisition
phyla
pili
populations
quorum sensing
reproduction
root hair growth
in seeds
shapes
single vs. plural
S-layers
in soil food web
species

strains
wall-less bacteria
bacterial blights
bacterial slime
bacterial soft rot
bacterial spots
bacterial symbionts
bacterivores
Bacteroidetes
bananas
barley
barley straw mulch
bases
basil
bayberry
beans
beech
beet
Beijerinck, Martinus
Bermuda grass
beta-carotene
Betaproteobacteria
binary fission
biobased systems
biocontrol
biodynamics
biofertilizers
biofilms
bioherbicide
bioluminescence
biomass
biopesticide
biopolymers
bioproducts
bioslime
biosynthesis
birch

blebbing
bokashi mixes
border cells
boron
Bosea
Botrytis
Bradyrhizobium
Bradyrhizobium japonicum
Brassica oleracea var. *alboglabra*
Brassica rapa var. *parachinensis*
brassinosteroids
Bromus tectorum
Bryobacter aggregatus
budding
butterflies
butyric acid

calcium
calcium hydroxide
calyptra
Canadian horseweed
cannabichromene (CBC)
cannabidiol (CBD)
cannabidiol-carboxylic acid
cannabigerol (CBG)
cannabinol (CBN)
cannabis
cannibal bacteria
capsule
carbohydrates
carbon
carbon dioxide (CO_2)
carbon fixation
carbonic acid (H_2CO_3)
carboxysomes
carrot

catalase
caterpillars
cell elongation
cell growth
cellulases
Cellulomonas
cellulose
cell walls
cereal crops
cheat grass
chelates
chelation
chemicals
chemoreceptors
chemotrophs
Chewings red fescue
chickweed
chitinases
Chloroflexi
chloroplasts
chromosomes
Chryseobacterium
Citrobacter
citrus canker
climate change
Clostridium
clover
coated seeds
coco coir
Coffea
coffee
coleoptile
coleorhiza
Comamonas
commercial formulations
competition
compost

compost teas
confocal laser scanning microscopy (CLSM)
conjugation
Conyza canadensis
copper
corn
cotton
cotyledons
creeping red fescue
crescentin
crop productivity
cucumber
curly dock
Curtobacterium
cyclosis
cytokinins
cytoplasm
cytoplasmic cycling
cytoskeletons

dandelions
DAPG (2,4-diacetylphloroglucinol)
Darwin, Charles
decay microbes
decomposers
deep soil disturbance
delta-9-tetrahydrocannabinol (THC)
differentiation zone
diffusion
diluted bleach solutions
disease resistance
DNA (deoxyribonucleic acid)
DNA testing
dormancy

earthworms

ectomycorrhizal fungi
education
elements
elongation zone
embryo
endobiome
endobiome interference
endocytosis
endophytes
endophytic bacteria
  discovery of
  education
  genera
  growing with
  growth stimulation
  host colonization
  host plants
  identification
  improved stress tolerance
  isolating
  legislation
  management practices
  market specialization
  mutual benefits
  nitrogen fixation
  phosphate solubilization
  phyla
  post-isolation hurdles
  research
  role in root development/growth
  role in root hair formation
  role of siderophores
  use in rhizophagy cycle
endophytic fungicides
endophytic insects

endosperm
endosphere
endospores
energy
Enterobacter
environmental conditions
enzymes
Epichloë festucae
epidermal cell
epidermis
epiphytic bacteria
Escherichia
Escherichia coli
esters
ethylene
Eukaryota (domain)
eukaryotes
exudates

facultative anaerobes
fatty acids
ferric iron
fertilizers
fimbriae
Firmicutes
fixed nitrogen
flagellum
flavonoids
Fragaria
fragrance
Frankia
Froelichia gracilis
fruits
FtsZ (filamenting temperature-sensitive mutant Z)
fungal hyphae
fungi

fungus-like water molds

Gammaproteobacteria
Gemmatimonadetes
gene expression
genera
gene regulation
genetic code
genetic testing
genotype
genotypic traits
genus
germination
gibberellic acid
gibberellins
glandular trichomes
glucanases
Gluconacetobacter
Gluconacetobacter diazotrophicus
glucose
Glycine max
Glycine max ‘Essex’
glycocalyx
glycolipids
glycolysis
glycopolymers
glycoprotein
grain crops
Gram, Christian
Gram-negative bacteria
Gram-positive bacteria
grape
grapefruit seed extract
grasses
green foxtail
growth regulators

growth stimulation
gymnosperms

Haber-Bosch process
halophytes
hard fescue
heavy metals
heirloom tomatoes
Helicobacter
hemicellulases
Herbaspirillum
Herbaspirillum seropedicae
herbicide
Holophaga foetida
homeostasis
Hordeum vulgare
hormones
host colonization
host plants
host resistance
humus
Humus Theory
hydrocarbons
hydrogen
hydrogen ions
hydrogen peroxide (H_2O_2)
hydrolysis
hydrophobic coating
hydroxides
hydroxyl ions
Hyphantria cunea
hypocotyl

ICC (1-aminocyclopropane-1-carboxylate)
immune responses

inclusions
indole-3-acetic acid (IAA)
induced-fit theory
infected hosts
Ingham, Elaine
inhibitors
inoculants
integuments
intestinal lining
iron

jasmine
jasmonic acids
Jerusalem artichoke
Johnston-Monje, David

Klebsiella
Klebsiella variicola
Korean natural farming (KNF)

lactic acid
Lactobacillus
Lactuca sativa
Laetisaria fuciformis
L-arginine
lawns
leaf spot pathogens
leaves
Leeuwenhoek, Antonie van
Legionellales
legislation
legumes
lettuce
L-form bacteria
Liebig, Justus von

ligand
lignin
Link, Heinrich Friedrich
Linnaeus, Carl
lipids
lipochitooligosaccharides
lipolytic activity
lipopeptides
Listeria
Lister Institute
Lolium arundinaceum
lycopene

magnesium
magnetite
magnetosomes
maize
management practices
manganese
market specialization
membranes
meristematic zone
meristem cells
messenger RNA (mRNA)
metabolites
methanol
methylobacteria
Methylobacterium
Methylobacterium extorquens
Microbacterium
microbes
microbial attractants
microbial noshing
microbiome
Micrococcus
Micrococcus luteus

microscopy
mineral acids
mitochondria
mitosis
molecules
monocot seeds
monocotyledons
mosquitoes
moths
mouse-ear cress
MreB
mucilage
mulches
Musa
mutual benefits
mutualists
mutually beneficial relationships
mycorrhizal fungi

NADPH (nicotinamide adenine dinucleotide phosphatase)
NADPH oxidase activity
natural selection
nematodes
Nitragin
nitrates
nitric acid
nitric oxide (NO)
nitrogen
nitrogen fixation
nodulation factors (NOD)
nodules
nomenclature
non-leguminous plants
non-psychoactive hemp
NPK
nucleic acids

nucleotides
nutrients
nut trees

oak
Ocimum basilicum
Olea europaea
oleaster
olive
one-sided commensalism
oomycetes
organic acids
organic gardening
osmosis
osmotic lysis
osmotic stress
oxidation
oxidative stress
oxygen

Paenibacillus
Paenibacillus durus
Pantoea
Pantoea agglomerans
parasitic relationships
passive diffusion
Pasteur, Louis
pathogenic bacteria
pathogenic fungi
pathogens
Paungfoo-Lonhienne, Chanyarat
peas
peat
pectin
Pectobacterium carotovorum
peptidoglycan

perennial ryegrass
pericycle
periplasm
periplasmic gel
periplasmic space
perlite
peroxidase
peroxynitrite
pesticides
pH
phenotype
phenotypic traits
phloem
phosphate
phosphate salts
phosphate solubilization
phospholipid membrane
phospholipids
phosphoric acid
phosphorus
photosynthesis
photosynthesizers
phyla
phyllosphere
phytobiomes
phytocannabinoids
phytohormones
pili
pineapple
pink-pigmented facultative methylotrophs (PPFMs)
Pinus sylvestris
Pisolithus arhizus
Planctomycetes
plant development
plant growth-promoting bacteria (PGPB)
plant growth-promoting hormones
plant regulation

plasma membranes
plasmids
Poa annua
polarity
polymers
polypeptide chains
polyphosphate granules
polyribosomes
polysaccharides
polysomes
pome fruit trees
poplar
Populus
porins
potash
potassium
potassium hydroxide
potato
potato black rot
powdery mildews
predators
prokaryotes
Propionibacteria
propionic acid
proteins
protein transcription
Proteobacteria
proton motive force
protoplasts
protozoa
pseudomonads
Pseudomonas
Pseudomonas fluorescens
Pseudomonas syringae
purple bacteria
pyoluteorin
pyrrolnitrin

pyruvic acid

quorum sensing

radicle
reactive oxygen species (ROS)
reduction
reproduction
research
respiration
rhizobia bacteria
Rhizobium
rhizophagy cycle
  bacterial symbionts in
  best practices
  breakdown of intracellular cell walls
  in cannabis plants
  concept of
  cytoplasmic cycling in
  disease resistance factors
  education
  endocytosis in
  host colonization
  mutual benefits
  nitrogen-fixing bacteria in
  nutrient uptake
  production of reactive oxygen species
  role of acids
  role of ethylene
  role of exudates
  role of pseudomonads
  role of roots
  root hair growth
  in soil food web

soil reuse for
stress tolerance factors
in tomato plants
use of compost teas
use of endophytic bacteria
use of ethylene
use of mucilage
use of slime
rhizoplane
rhizosphere
Rhodococcus
ribosomes
rice
rice cutgrass
RNA (ribonucleic acid)
root cap
root cell elongation
root cells
root development/growth
root hairs
roots
root vegetables
root zones
rosary pea
roses
rototilling
rubisco
Rumex crispus
rust
rutabaga

Saccharibacteria
Saccharomyces cerevisiae
Saccharum officinarum
salicylates
salicylic acid

salinity
Salix
Salmonella
saprophytic bacteria
scab
Sclerotinia homoeocarpa
Scots pine
seaweed
seed leaves
seeds
  bacteria in
  coated seeds
  embryo
  germination
  microbiome
  seed coat
  sterilizing
  storage
  structure
selenium
Serratia
Setaria viridis
sex pili
siderophores
signaling
single-cell endodermis
Sinorhizobium
S-layers
slime
slime layers
sludge
snakecotton
sodium
sodium hydroxide
soil
soil food web

soil-less mixes
soil microbiota
soil reuse
soil sterilization
Solanum lycopersicum
Solanum tuberosum
sorghum
soybean
species
spheroplasts
Sphingobacteriales
Sphingomonas
Sphingopyxis
squash
staph
Staphylococcus
Staphylococcus aureus
statocysts
strains
strawberry
strep
Streptococcus
Streptomyces scabies
stress
Striga hermonthica
strigolactones
strophiolar cleft
sugarcane
sugars
Suillus variegatus
sulfur
sulfuric acid
superoxide
symbiosis
symbiotic relationships

tall fescue
tannins
Taraxacum officinale
tegmen
teichoic acid structures
temperature
teosinte
terpenes
terroir
testa
thermophiles
tobacco
tomato
toxins
traits
trees
trichoblast
trichomes
Trifolium repens
truffles
Tull, Jethro
turf grasses
turgor pressure

vegetables
vermicompost
vermiculite
Verrucomicrobia
vinegar solutions
viruses
vitamin A
vitamin B12
vitamin D
vitamin E
vitamins

wall-less bacteria
webworm
weeds
wheat
White, James Francis
willow

Xanthomonas
Xanthomonas campestris
Xanthomonas campestris pv. *poae*
xylem

yeast

Zea mays
zinc

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